INDIAN PHYTOPATHOLOGY

VOLUME VI 1953 *NUMBER 1*

(ISSUED APRIL, 1954)

PUBLISHED FOR

INDIAN PHYTOPATHOLOGICAL SOCIETY
PUSA BUILDINGS, NEW DELHI-12
INDIA

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FUNGITOXICITY OF SULFUR-BRIDGED COMPOUNDS*

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(Accepted for publication, December 31, 1953)

The mechanism of sulfur toxicity has intrigued the scientifically curious ever since sulfur was found to have "pest-averting" qualities. Probably the most extensive researches have been conducted by McCallan and Wilcoxon (1931) and their colleagues at the Boyce Thompson Institute.

In our studies on mechanisms of fungicidal action, we have had occasion to investigate many types of organic sulfur compounds.

In this paper, we shall present results of the researches on those organic compounds that contain a sulfur bridge. We trust that the results may shed a few lux of light on some dark corners of the complex problem.

We shall not include any data on derivatives of dithio acids (-CSS- compounds) because these constitute a whole continuing subject in themselves (Barratt and Horsfall, 1947).

MATERIALS AND METHODS

Our test method is the "slide-germination" method of the American Phytopathological Society (1943) slightly modified. Fungitoxicity is measured in terms of the ability of a compound to inhibit germination of spores. The test compound is dissolved in a suitable solvent, usually acetone at four concentrations—10, 1.0, 0.1, and 0.01 grams per liter. A 0.2 ml. aliquot is pipetted with a graduated syringe into small cylindrical depressions (15 mm D, 3 mm deep) in glass microscope slides—so-called culture slides.

The solvent is allowed to evaporate leaving a deposit of the test compound in the bottom of the depression and then 0.4 ml. of spore suspension is pipetted in with a separate graduated syringe. The deposits corresponding to the four dosages are 1300, 130, 13 and 1.3 μgm/cm^2, respectively. The spore concentration is adjusted to give 40 to 50 spores per low power field of the microscope. The amount pipetted fills the depression level full and this avoids optical distortion when counts are made of the germinated spores. It should be mentioned that this technique will not adequately appraise the toxicity of compounds with high vapor pressures. In other words, volatile toxic compounds will appear non-toxic.

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*Contribution from The Connecticut Agricultural Experiment Station, New Haven, Conn. The research has been conducted in co-operation with the Crop Protection Institute. We wish to thank Dr. C.H. MacBurney and W.E. Craig of the Rohm and Haas Company for providing numerous test compounds and for much stimulating discussion. We wish to thank also Mrs. Eleanor Moquet, Mrs. Barbara Kluck and Mrs. Joan Szabo for the excellence of their assistance with the research.

The slides are incubated overnight in moist chambers made of petri dishes or other glass containers, and the proportion of non-germinated spores is recorded in the morning. The test organisms are *Stemphylium* (formerly *Macrosporium*) *sarcinaeforme* and *Monilinia* (formerly *Sclerotinia*) *fructicola.*

Experimental Data

The four doses give five grades of toxicity—non-toxic means few spores inhibited at 1300 μgm/cm^2. Weakly toxic means all or almost all spores killed at the highest dose only. Toxic means spores killed at both high doses. Strongly toxic means spores killed at the three highest doses. And very strongly toxic means spores killed at all four doses. The data are given in Table I.

Differential Action on Fungi

Even a brief study of the table will show that the sulfur-bridged compounds tested are more toxic to *Monilinia* than to *Stemphylium*. A simple count reveals that 29 are more toxic to *Monilinia*, whereas only 11 are more toxic to *Stemphylium.*

In the first report on *Stemphylium* as a laboratory test organism, it was noted that it is not sensitive to elemental sulfur (Horsfall, 1930). McCallan and Wilcoxon (1931) later showed that *Monilinia* is far more sensitive to elemental sulfur than *Stemphylium*. We find here that *Monilinia* is also more sensitive to sulfur-bridged compounds than *Stemphylium.*

That being so, the major emphasis in the paper will be on the reactions of *Monilinia*. The reactions of *Stemphylium* will be considered only where they seem to differ significantly. As Horsfall and Rich (1951) have shown, *Stemphylium* is much more sensitive to acids than *Monilinia.* In fact, most of the 11 sulfur-bridged compounds to which *Stemphylium* is especially sensitive are acids.

Simple Thioethers

In general the simple thioethers were without significant toxicity to either test fungus. Those tested were *n*-butylsulfide (No. 456), *n*-propylsulfide (No. 560), allylsulfide (No. 561), octylsulfide (No. 1031), octyldisulfide (No. 1036), decylsulfide (No. 1032), benzyldisulfide (No. 466), and ethyltrithioformate (No. 1363).

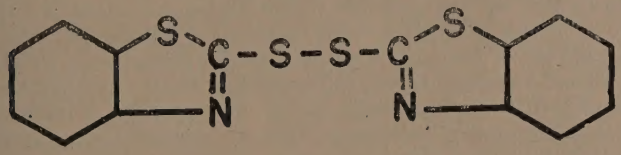
It turns out that this group of negative compounds was negative for different reasons. The butyl, propyl, and allyl sulfides and ethyl-trithioformate are volatile. Hence, there was no residue to test. A test of the gas phase showed that these compounds are fungitoxic. In this test 0.1 ml was placed in a microbeaker in a petri dish with untreated spores. The spores failed to germinate.

The other four compounds were not toxic by either test. A final test was to put spores on an undried quantity of test compound. They were not inhibited. It seems safe to conclude that poor permeation was responsible for the negative results with octylsulfide, octyldisulfide and decylsulfide. The molecules of these seem either too big or too predominantly lipid soluble. Each has from 16 to 20 carbons. Many persons

(Horsfall, 1945) have suggested that the optimum number of carbons is near 11. The active compounds comprise 8 or 10 carbons. Benzyl disulfide will be discussed below.

Three thiophene derivatives were investigated 3-(1,1,3,3-tetramethyl-butylmercapto) thiophene (No. 1017), 3-(isopropylmercapto) thiophene (No. 1018), and 3-(allylmercapto) thiophene (No. 1019).

The first two were bland on the spores and non-volatile. The third was toxic to *Monilinia*. Here also, permeation is probably involved. Compounds with branched chains in general are less fungitoxic than unbranched homologues. It seems likely that the warts on the aliphatic tails of compound Nos. 1017 and 1018 reduce the amount of permeation. The hydrocarbon group of compound No. 1019 is not branched ; the compound presumably permeates more readily and is more toxic. No. 1018 and No. 1019 differ in molecular weight by only two hydrogen atoms, yet No. 1019 is toxic, No. 1018 is not ; No. 1018 has a branched chain, No. 1019 has not.



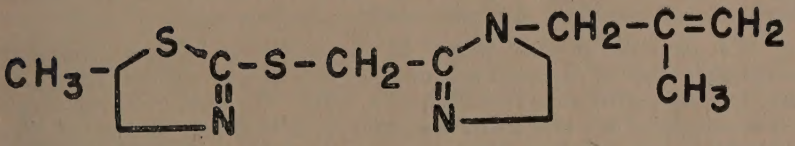


Figure I—Two heterocycles with sulfur bridges

Upper 2,2'—Dithiobis (benzothiazole) No. 134

Lower 5—Methyl—2—(1—(2—methylallyl)—2—imidazoline—2—yl) methyl-mercapto—2—thiazoline. No. 727

Two other heterocyclic compounds show a very interesting effect. These are illustrated in Figure 1. One of these (No.134) contains a disulfide bridge between two benzothiazole nuclei. It is non-fungitoxic. The other (No. 727) contains a —S—CH_2—bridge between a thiazoline and an imidazoline nucleus. It is fungitoxic, but not just to *Monilinia* as the thiophene compounds just discussed. That the latter compound is toxic to *Stemphylium* suggests that the sulfur bridge is not the sole fungitoxic moiety.

It is difficult to say whether the active group for *Stemphylium* is the thiazoline or the imidazoline nucleus or both.

We lean slightly toward the view that the compound owes its fungitoxicity to the imidazoline nucleus. Wellman and McCallan (1946) have shown that imidazoline is fungitoxic if it carries a long hydrocarbon tail in

the 2-position. Rich and Horsfall (1952) have adduced evidence that the hydrocarbon tail functions as a lipophilic group to drive the compound through the semipermeable defenses of the cell. It seems probable that the 2-substitution on the imidazoline moiety of compound No. 727 may function also as a lipophilic group that promotes permeation. If so, the sulfur bridge is only an incidental part of the molecule and may well contribute no activity.

Two other thioethers without additional reactive groups are interesting. These are diphenyl sulfide (No. 493) and diphenyl disulfide (No. 487). These are ranked toxic and strongly toxic, respectively to *Monilinia*. They are far more toxic than would be indicated by their nearest available aliphatic counterparts, octylsulfide (No. 1031) and octyldisulfide (No. 1036). The resonating rings of the diphenyl sulfides must be important to toxicity.

The effect of resonating rings on fungitoxicity may be appraised in the light of modern electronic theory. According to Gilman (1943) resonant energy is energy in excess ot the sum of the energy of the separate bonds making up the molecule. The Arrhenius activation theory states that excess molecular energy seems to activate molecules and produce a more rapid rate of chemical reaction. As this energy of activation is exponentially related to reaction velocity according to the Arrhenius equation, small amounts of excess molecular energy may greatly increase rates of reaction. Hence, resonating structures, such as benzene rings, thiophene rings, and other conjugated systems may serve as powerhouses to activate potentially reactive groupings. If fungitoxicity is dependent on one or more chemical reactions, as it must undoubtedly be in most cases, then any property of the fungitoxic molecule which would increase the rate of chemical reactions must, perforce, enhance fungitoxicity.

The converse is also true. If the sulfur is insulated from the resonating rings of diphenyl sulfide by even a single carbon group, the toxicity disappears (benzyl disulfide, No. 466). It would be instructive to test a compound in which sulfur was used to bridge two saturated rings which could not resonate. Such a compound was not available.

Two resonating rings alone are not enough for toxicity, either, because diphenyl itself is not toxic to spores of either fungus as Horsfall, Chapman and Rich (1951) have reported. Also, oxidation of the sulfur of diphenyl sulfide to sulfone (No. 476) quenches the toxicity. Apparently, reduced sulfur is necessary. Perhaps sulfur in the bridge is not absolutely necessary because phenyl ether (No. 1382) is weakly toxic, not as toxic, however, as the sulfur analogue. One cannot conclude, however, that just any bridge between two rings is effective because diphenylmethane and diphenylamine are inactive. (See Horsfall, Chapman, and Rich, 1951).

Effect of the Carboxyl Group

A carboxyl group converts a non-toxic sulfur bridged dialkyl compound into a fungitoxic compound.

The synergistic effect of carboxyl on alkyl compounds is best witnessed by octylmercapto acetic acid (No. 1167), β-octylmercaptopropionic acid (No. 1168), β-nonylmercaptopropionic acid (No. 1171),

θ – propylmercaptopelargonic acid (No. 1170), and 5 – methylmercapto – 2 – pentenoic acid (No. 1650). The conversion of the latter to the sodium salt (No. 1651) quenches the toxicity. This is probably a case of poor permeation, however, as will be discussed below.

It may be noted that the number of carbons between the sulfur bridge and the carboxyl group is immaterial; neither does it matter that one double bond occurs.

One of the interesting series for studying the relation of sulfur bridging between a benzene ring and an aliphatic acid (acrylic) is based on compound No. 1605, C_6H_5-S-CH=CHCOOH. It is strongly toxic to both organisms. It is more toxic than benzoic acid (No. 831). This seemed at first like a worthy example of the accelerating effect of a sulfur bridge. The illusion was soon blasted because compound No. 1484 (*o*-chlorocinnamic acid) is equally as toxic and it lacks the sulfur bridge.

We had available for test through the kindness of Dr. C. P. Lo of Rohm and Haas Company a series of derivatives of No. 1484—o-chlorocinnamic acid. These compounds were β – phenylmercaptoacrylic acid (No. 1605), α – (*p* – chlorobenzylmercapto) cinnamic acid (No. 2572), α – (*p* – chlorobenzylmercapto) – β – (2 furyl) acrylic acid (No. 2573), α – carboxymethylmercaptocinnamic acid (No. 2574), *p*-chlorobenzylidenebisthioglycolic acid (No. 2616), potassium – α – (*p*-chlorobenzylmercapto) cinnamate (No. 2618), and α – mercapto – 2, 4 – dichlorocinnamic acid (No. 2619). These compounds were substituted variously, but chiefly on the α – carbon of the acrylic acid to give a sulfur bridged moiety.

Only two of the analogues (Nos. 2574 and 2616) showed any changed toxicity pattern and both reduced markedly the activity on *Monilinia*. Both contain two carboxyl groups instead of the usual one. Apparently, a second carboxyl group cancels the activity of the first. One of them (No. 2616) contains two-S-CH_2-COOH groups and its toxicity to *Stemphylium* seems to be enhanced.

The striking feature of the compounds that are active on *Monilinia* is the alternating double bonds between the—COOH and the ring, assuming that a sulfur bridge acts isosterically with—CH=CH—. Since this is equivalent to one vinyl unit, the presence or absence of the sulfur bridge is unimportant. Apparently, resonating energy is important here also in the creation of fungitoxicity.

The only exception is compound No. 2574. It is inactive on *Monilinia* despite the alternating double bonds. It contains two carboxyl groups, however, and they cancel each other out. As Horsfall and Rich (1951) have shown high toxicity on *Monilinia* requires high lipid solubility. The extra polar group (carboxyl) on No. 2574 probably makes the compound so water soluble that permeation into *Monilinia* is reduced, and, hence, toxicity is reduced.

There are three other sulfur-bridged organic acids that involve a benzene ring. They all involve two carboxyl groups, as well.

The three compounds are *o*, *o'*-dithiobenzoic acid (No. 1113), *o*-(carboxymethylmercapto) benzoic acid (No. 1111), and α – (2 – carboxy-

phenylmercapto) propionic acid (No. 1112). These are weak on *Monilinia* but strong on *Stemphylium*. These compounds also contain two carboxyl groups.

These three compounds differ from the ones discussed just previously in that at least one carboxyl is attached to a benzene ring. In this respect they can be compared with benzoic acid (No. 831). All have the second carboxyl group separated from the ring by a sulfur-hydrocarbon linkage.

So far as an analogy with benzoic acid goes, that acid is more toxic to *Monilinia* and less toxic to *Stemphylium* than any of the three compounds under discussion. Apparently, the extra carboxyl group enhances the toxicity to *Stemphylium* and reduces the toxicity to *Monilinia*, just as in the other compounds.

Similary *o*-phthalic acid (No. 673) which can be considered as benzoic with a second carboxylic group, is less toxic to *Monilinia* and more toxic to *Stemphylium* than benzoic acid itself (No. 831).

In one example of an aliphatic acid, azelaic (9 carbon, 2 acid) is less toxic to *Monilinia* than pelargonic (9 carbon, 1 acid). Toxicity to *Stemphylium* is about the same. Kitajima and Kawamura (1931) show that an extra carboxyl on a fatty acid reduces the fungitoxicity to wood destroying fungi.

One would gather from all this that the sulfur bridges in compounds 1111, 1112, and 1113 are not involved in the cancellation effect of a second carboxyl.

A carboxyl group also synergizes a sulfur bridge between a thiazole and an alkyl group, 2-carboxymethylmercaptobenzothiazole (No. 1744).

Two analogues of this compound are useful in helping to decide the relative importance for toxicity of the sulfur bridge and the acid moiety. The following points suggest that the acidity is not the important consideration. (1) *Stemphylium* is much more sensitive to acids than *Monilinia* and yet compound No. 1744 is about as toxic to *Monilinia* as to *Stemphylium*. (2) The acidity of 2-carboxymethylmercaptobenzothiazole (No. 1744) can be quenched by making the ester, which is 2-carbethoxymethylmercaptobenzothiazole (No. 1722). The ester is much less toxic to *Stemphylium* than the free acid as expected if acidity were involved, but is *more toxic* to *Monilinia*, and *Monilinia* seems to be the significant organism in the tests of sulfur bridges. The extra toxicity of the ethyl ester to *Monilinia* is probably due to better permeation through the semipermeable membrane of the spore. The ester ionizes less than the free acid and it is generally true that the less the ionization the better the permeation and the greater the toxicity. (3) The acidity of an acid can also be quenched by conversion to the sodium salt, No. 1660, or to the potassium salt, No. 1413. They are only weakly toxic to either organism. Here again the governing factor in toxicity seems to be ionization and permeation. Ionization would follow this sequence, salt $>$ acid $>$ ester, and toxicity follows the reverse sequence, ester $>$ acid $>$ salt. (4) The acidity of No. 1744 may be reduced also by conversion to the amide and this is No. 1723. This compound like the ester is less fungitoxic to *Stemphylium* than the free acid, and about equally as toxic to *Monilinia*. The fact that

the amide is less toxic to *Monilinia* than the ester will be discussed below under the quenching effect of amino groups.

If data on the amide and the ester of No. 1744 are considered together, one is led to suspect that it is the ketone group that is responsible for toxicity in the acids. The significance of the ketone will appear again below when the subject of $-SCN$, and $-S-CCl_3$ groups are discussed.

Quenching effect of $-NH_2$

An $-NH_2$ group usually quenches the toxicity of a sulfur bridged carboxy compound. The sulfur containing amino acid, methionine, is a familiar example. Not only is the sulfur non-toxic, the compound is essential for the life of most organisms. Its essentiality for our test fungi has not been ascertained, however.

DL-methionine (No. 1586) and three N-substituted analogues of it were run. The analogues were 2-carboxyethyl (No. 2223), 2-cyanoethyl (No. 1261), and bis (2-cyanoethyl) (No. 1260). It is interesting that methionine itself and the mono-substituted compounds were bland. They exerted no toxicity. If, however, both amino hydrogens were replaced (No. 1260) the compound showed weak toxicity to *Monilinia* and good toxicity to *Stemphylium*. It would appear from the differential effect on *Stemphylium* that No. 1260 owes its effect to the carboxyl group and, hence, that the quenching effect of the $-NH_2$ is on the carboxyl group and not on the sulfur bridge.

Another N-substituted amino acid, valine, was tested. The compound is N-benzoyl-β-(benzylmercapto) DL valine (No. 2117). It contains, of course, an NH— group near the carboxyl group. Even though only one amino hydrogen has been replaced, the compound acts as if both were substituted. It is toxic to *Stemphylium* but only weakly toxic to *Monilinia*. The substitution is benzoyl and this places a C=O group on the other side of the —NH— group from the carboxyl. Apparently, the —NH— can reduce the effectiveness of only one of the two nearby oxidized groups.

Data were available on two other compounds that contain a sulfur bridge and a carboxyl group plus an $-NH_2$ group. These are S-methylthioammeline (No. 1518), S-carboxymethylthioammeline (No. 1551) and β-(guanylmercapto)-propionic acid (No. 2156). None was fungitoxic. It is well to recall here, also, that the amide (No. 1723) of No. 1744 is less toxic than the ester (No. 1722). This seems to be due to a quenching effect of the $-NH_2$ on toxicity of the carbonyl group.

It seems reasonable to deduce, then, that $-NH_2$ in the molecule quenches the toxicity that derives from the presence of a carboxyl group in a sulfur bridged molecule.

Effect of the thiocyanate group ($-SCN$)

The thiocyanate group is usually considered as a toxophore in biologically active compounds. Its activity in our tests varied greatly with the compound in which it occurred, however. Three salts were essentially non-toxic, ammonium thiocyanate (No. 559), sodium thiocyanate (No. 558), and guanidine thiocyanate (No. 718). It is probable that these are non-toxic for the same reason that the salts of the carboxy acids

discussed above are non-toxic. They probably ionize too readily for good permeation.

Several thiocyanocarboxylic esters were generally toxic, especially to *Monilinia*. These were phenyl-β-thiocyanoethylcarbonate (W—10), allylthiocyanoacetate (H—358), and carbitol ester of β—thiocyanopropionic acid (H—383). The results with these esters agree with the results with the other esters of carboxylic acids discussed above and they suggest that sulfur in the —S—CN group acts much as it does between other carbon-containing groups.

Another active compound on both organisms is thiocyanoacetone (No. H-394). This seems to depend for its activity on the presence of a carbonyl and a sulfur bridge in the molecule as already mentioned above on esters.

Three thiocyano compounds contain amino groups; one of these contains a tertiary amine *p*-thiocyanodiethylaniline (No. H—166). It is toxic to *Monilinia* but bland to *Stemphylium*. The other, benzylidene-*p*-thiocyanoaniline (H—155), is strongly toxic to both organisms. Bis (4-thiocyanophenyl) amine (No. 1828) shows an unusual toxicity picture especially for *Monilinia*. It shows what we call a TMTD curve (See Barratt and Horsfall, 1946), so called because it resembles the toxicity curve for tetramethylthiuramdisulfide. That is, the spore inhibition is high as expected for a high dose. Spore inhibition declines with dosage as expected and then it unexpectedly rises again as dosage continues to go down. This result has been found in three separate tests. This compound contains two thiocyano groups. Still ethylene thiocyanate (No. 554) also contains two thiocyano groups. It is toxic, but it displays no TMTD curve. Of course, the two —SCN groups in the latter compound are separated by an ethylene bridge, the other by a diphenylamine bridge. Also the sulfur atoms of No. 1828 are attached to resonating rings.

The most active thiocyanate compound is 2,4-dinitrophenylthiocyanate (No. 894). We are unhappy over this compound because we screened it in February, 1943, and learned of its excellent fungitoxic properties. In fact, we found that it is reasonably resistant to rainfall, but unfortunately, we did not carry it to the field. It was developed, however, as an active fungicide in Germany during the war under the common name of Nirit. Muncie and Hatfield (1951) have shown that it gives good control of late blight of potato in the field.

The fungitoxicity of the compound seems to depend upon the occurrence of an $-NO_2$ and an —S—CN on the same ring.

Effect of trichloromethylthio group ($-S-CCl_3$)

Kittleson (1952) published that $-S-CCl_3$ would drastically improve the fungitoxicity of compounds containing the following group $-\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{N}}-\overset{O}{\overset{\|}{C}}-$ if it were substituted for the amino hydrogen. The compound chosen for development was N – trichloromethylthiotetrahydrophthalimide (No. 810). This has been given the common name of captan. It should be noted that captan contains a carbonyl fairly closely juxtaposed

to a sulfur bridge as in compounds previously discussed. This is a very active and promising new fungicide.

The $-S-CCl_3$ group is a very interesting substituent and it will constitute the subject of a separate communication.

One wonders if the use of $-SCN$ would not accomplish the same result as $-S-CCl_3$. Dr. R.L. Wain of Wye College, England, has suggested in conversation with the writers that the activity of the $-S-CCl_3$ grouping is associated with the fact that it is strongly electronegative. If so, the $-SCN$ should be a suitable substitute for it. In any case, Wain's hypothesis gains some credence from the fact that N – nitrosophthalimide has been patented as an active fungicide. The nitroso group is a well known electronegative group.

Effect of substituents on diphenyl sulfide or diphenyl disulfide

As noted earlier, diphenyl sulfide (No. 493) is reasonably toxic to *Monilinia*. Diphenyl sulfoxide (No. 479) is about as toxic, but if the sulfur bridge is completely oxidized to the sulfone (No. 476), the toxicity to *Monilinia* is completely quenched. It is well known that sulfonamides are essentially non-fungitoxic (Horsfall, 1945). Here also the sulfur bridge is completely oxidized.

We have already seen that the insertion of a carboxyl group on each ring in the positions ortho to the disulfide linkage (No. 1113) quenches the toxicity of diphenyl disulfide (No. 487). Likewise, insertion of two nitro groups in the same positions (No. 472) also quenches the toxicity. If however, the nitro groups are moved farther from the sulfur—to the *meta* (No. 808) or *para* (No. 488) positions, toxicity is restored to *Monilinia*. The compound with the *meta* nitro substitution is the only one of the three that is toxic at all to *Stemphylium*. Even so the results are anomalous. The toxicity is essentially the same for all doses and this has been checked in three separate experiments over three years. The meta compound is sold under the name of "Nitrophenide" as a drug for coccidiosis of chickens.

In contradistinction to the quenching effect of carboxyl and nitro groups, insertion of hydroxyl (Cr 305) and amino (No. 741) groups in the two *ortho* positions greatly enhances the toxicity of diphenyl sulfide. Bis (2-hydroxy-5-chlorophenyl) sulfide (No. Cr 305) was introduced as a fungicide by Rich and Horsfall (1950). It need only be mentioned here as it will constitute the subject of a separate paper. Schraffstatter *et al.* (1949) have worked with this compound as an orally administered therapeutant for certain fungus diseases of man. They hold that it may function to chelate the metals needed by the fungus. Chelation is a toxic mechanism that was proposed by Zentmyer (1944). Goldsworthy and Gertler (1949) reported that bis (2-aminophenyl) sulfide (No. 741) is a fungicide. This compound also probably can chelate metals.

Discussion and summary

A sulfur bridge occurs in the well known dithiocarbamate fungicides, but it is a sulfur bridge between carbon atoms that is concerned in this paper. Several interesting points have developed during a study of sulfur bridges.

(1) *Monilinia* is more sensitive than *Stemphylium* to compounds with a sulfur bridge between carbon atoms and, hence, *Monilinia* makes a more sensitive indicator organism.

(2) Several sulfur bridged compounds lack fungitoxicity. This appears to be due to decreased permeation. For example, butyl, propyl, and allyl sulfides are toxic, but octyl and decyl are not. Apparently the molecule of octyl or decyl sulfide is too large or insufficiently hydrophilic to permeate properly.

On the other hand, the molecule can also be too hydrophilic for good activity. A carboxymethylmercapto group lends toxicity, but two carboxymethylmercapto groups reduce toxicity to *Monilinia*. The second polar group probably makes the compound too hydrophilic.

The molecule can be of an improper shape too. 3-(isopropylmercapto) thiophene is non-toxic whereas 3-(allylmercapto) thiophene is toxic. It seems reasonable to assume that the difference lies in the shape of the side chain. The two methyl branches on the isopropylmercapto group seem to impede permeation.

(3) In most of the active compounds, the sulfur occurs between two active groups such as $-CN$, $-NO_2$, $-CCl_3$, $-CO-$, $-COOH$, $-COO$ ester. A benzene ring either sides also gives an active fungicide. The striking feature is that all of these groups are electronegative. Presumably the electronegative groups increase the reactivity of the $-S-$ bridge. If an electropositive group like $-NH_2$ occurs in the molecule, it is likely to cancel the fungitoxic effect due to an electronegative group.

(4) The sulfur bridge in at least two types of structures must be in the reduced form or it is inactive. Diphenyl sulfide is toxic; diphenyl sulfone is not. β—Nonylmercaptopropionic acid is fungitoxic whereas β—nonylsulfonylpropionic acid is not. No sulfonamide has been found to be fungitoxic. This action of reduced sulfur recalls to mind the work of Marsh (1929) and of McCallan and Wilcoxon (1931) who showed evidence that the fungicidal action of sulfur is due to reduced sulfur as hydrogen sulfide. McCallan and Wilcoxon (1931) explained an old observation (Horsfall, 1930) that *Stemphylium* is not sensitive to elemental sulfur. They showed that the fungus was not able to produce enough H_2S from sulfur to kill itself. *Monilinia* can.

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TABLE I

Fungitoxicity of sulfur-bridged compounds

No.	Name	Source of Cpd.**	Percentage of spores not germinating							
			S. sarcinaeforme				*M. fructicola*			
			1300*	130*	13*	1·3*	1300*	130*	13*	1·3*
W-10	Phenyl-β-thiocyanoethylcarbonate	R & H	100	0	0	0	100	100	0	0
H-155	Benzylidene-*p*-thiocyanoaniline	R & H	100	100	85	0	100	100	34	0
H-166	*p*-Thiocyanodiethylaniline	R & H	72	0	0	0	100	100	71	0
H-358	Allylthiocyanoacetate	R & H	100	0	0	0	100	100	0	0
H-383	Carbitol ester of β-thiocyanopropionic acid	R & H	100	100	—	—	100	100	—	—
H-394	Thiocyanoacetone	R & H	100	50	0	0	100	50	0	0
134	2,2'-Dithiobis (benzothiazole)	R T V	0	0	0	0	100	0	0	0
Cr-305	Bis(2-hydroxy-5-chlorophenyl) sulfide	R & H	100	92	92	7	100	100	100	80
456	n-Butylsulfide	E K	0	0	0	0	0	0	0	0
466	Benzyldisulfide	E K	0	0	0	0	50	0	0	0
472	Bis (2-nitrophenyl) disulfide	E K	0	0	0	0	0	0	0	0
476	Diphenylsulfone	E K	0	0	0	0	0	0	0	0
479	Diphenylsulfoxide	E K	100	50	0	0	100	100	0	0
481	Bis (4-bromophenyl) sulfone	E K	0	0	0	0	0	0	0	0
487	Diphenyldisulfide	E K	97	46	0	0	100	100	100	45
488	Bis (4-nitrophenyl) disulfide	E K	57	21	0	0	100	100	100	48
493	Diphenylsulfide	E K	0	0	0	0	100	100	0	0
554	Ethylenethiocyanate	E K	100	100	45	0	100	100	14	0
558	Sodium thiocyanate	E K	0	0	0	0	100	0	0	0
559	Ammonium thiocyanate	E K	100	0	0	0	0	0	0	0
560	n-Propylsulfide	E K	—	0	—	—	—	0	—	—
561	Allylsulfide	E K	42	0	0	0	100	0	0	0
673	*o*-Phthalic acid	E K	100	100	100	2	100	1	12	0

718	Guanidine thiocyanate	E K	100	0	0	0	100	67	0	0
727	5-Methyl-2-[1-(2-methylallyl)-2-imidazoline-2-yl]-methylmercapto-2-thiazoline	CBCC	100	100	7	0	100	100	83	16
741	Bis(2-aminophenyl) sulfide	CBCC	100	100	100	39	100	100	100	22
808	Bis-(3-nitrophenyl) disulfide	A C	56	56	67	44	100	100	100	45
810	N-(trichloromethylthio),1,2,3,6-tetrahydrophthalimide	S.O.N.J	100	100	100	48	100	100	100	100
		E K	100	100	0	0	100	100	6	0
831	Benzoic acid	R & H	100	100	100	?	100	100	100	?
894	2,4-Dinitrophenylthiocyanate	CBCC	0	0	0	0	50	0	0	0
1017	3-(1,1,3,3-Tetramethylbutylmercapto)thiophene	CBCC	0	0	0	0	50	0	0	0
1018	3-(Isopropylmercapto)thiophene	CBCC	7	0	0	0	100	99	4	0
1019	3-(Allylmercapto)thiophene	CBCC	0	0	0	0	0	0	0	0
1031	Octylsulfide	CBCC	0	0	0	0	0	0	0	0
1032	Decylsulfide	CBCC	0	0	0	0	0	0	0	0
1036	Octyldisulfide	CBCC	100	100	100	0	100	28	0	0
1111	*o*-(Carboxymethylmercapto) benzoic acid	CBCC	100	100	28	0	100	0	0	0
1112	α-(2-Carboxyphenylmercapto) propionic acid	CBCC	100	97	23	0	100	0	0	0
1113	*o*,*o*'-Dithiobenzoic acid	CBCC	100	100	61	0	100	100	15	0
1167	Octylmercaptoacetic acid	CBCC	100	100	18	0	100	100	100	10
1168	β-Octylmercaptopropionic acid	CBCC	100	93	8	0	100	100	100	1
1170	θ-Propylmercaptopelargonic acid	CBCC	100	98	28	0	100	100	100	0
1171	β-Nonylmercaptopropionic acid	CBCC	100	100	58	0	98	8	1	4
1260	N,N-Bis(2-cyanoethyl)DL-methionine	CBCC	0	0	0	0	0	0	0	0
1261	N-(2-cyanoethyl)DL-methionine	CBCC	2	0	1	0	100	34	7	0
1363	Ethyltrithioformate	E K	69	5	0	1	100	46	7	6
1382	Phenyl ether	R & H	74	27	3	2	78	24	1	1
1413	Potassium salt of 2-carboxymethylmercaptobenzothiazole	CBCC	100	100	98	1	100	100	36	14
1484	*o*-Chlorocinnamic acid									

TABLE I (*Cont.*)—*Fungitoxicity of sulfur-bridged compounds*

No.	Name	Source of Cpd.**	Percentage of spores not germinating							
			S. sarcinaeforme				*M. fructicola*			
			1300*	130*	13*	1·3*	1300*	130*	13*	1·3*
1518	S-methylthioammeline	R & H	0	0	0	0	0	0	0	0
1551	S-carboxymethylthioammeline	R & H	67	0	0	0	0	0	0	0
1586	DL-methionine	R & H	1	0	0	0	5	1	0	0
1605	β-Phenylmercaptoacrylic acid	R & H	100	100	20	0	100	100	54	0
1650	5-Methylmercapto-2-pentenoic acid	R & H	100	100	87	0	100	100	0	0
1651	Sodium-5-methylmercapto-2-pentenoate	R & H	44	20	4	3	18	8	0	0
1660	Sodium salt of 2-carboxymethylmercapto-benzothiazole	R & H	100	0	0	0	100	0	0	0
1722	2-Carbethoxymethylmercaptobenzothiazole	R & H	100	76	5	55	100	100	94	100
1723	2-Carbamidomethylmercaptobenzothiazole	R & H	100	8	8	4	100	100	16	3
1744	2-Carboxymethylmercaptobenzothiazole	R & H	100	100	54	0	100	100	0	0
1828	Bis(4-thiocyanophenyl) amine	CBCC	0	0	13	65	81	49	86	94
2117	N-benzoyl-β-(benzylmercapto) DL valine	CBCC	100	100	63	0	37	32	15	0
2156	β-(Guanylmercapto)propionic acid	CBCC	0	0	0	0	0	0	0	0
2223	N-(2-carboxyethyl)-DL methionine	CBCC	100	0	0	0	16	0	0	0
2572	α-(p-Chlorobenzylmercapto)cinnamic acid	R & H	100	66	0	0	100	100	16	0
2573	α-(p-Chlorobenzylmercapto)-β-(2-furyl) acrylic acid	R & H	100	58	0	0	100	100	17	0
2574	α-Carboxymethylmercaptocinnamic acid	R & H	100	100	79	0	100	0	0	0
2616	p-Chlorobenzylidenebisthioglycolic acid	R & H	100	100	100	100	100	20	0	0
2618	Potassium-α-(p-chlorobenzylmercapto)cinnamate	R & H	100	0	0	0	100	0	0	0
2619	α-Mercapto-2,4-dichlorocinnamic acid	R & H	100	100	34	0	100	100	0	0

** Code for sources of compounds: AC—American Cyanamide Co., Stamford Connecticut; CBCC—Chemical—Biological Coordination Centre of the National Academy of Science, National Research Council; EK—purchased Eastman Kodak Co., Rochester, New York; SONJ—Standard Oil Co. of New Jersey, Elizabeth, New Jersey.

* Dosages in micrograms per cm^2 of surface.

A NEW *HELMINTHOSPORIUM* ON WHEAT

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(Accepted for publication, July 1, 1953)

Wheat (*Triticum aestivum*) which is extensively grown in Bombay State was first reported to have been attacked by this disease in 1946, from Baramati. In successive years, the disease was also reported from Kaira, Ahmedabad and Panch Mahals. Pusa 4 in Poona and Niphad 4 in other districts suffered from this disease to the extent of about 50 per cent in some years.

Several species of *Helminthosporium* such as *H. sativum* Pammel, Kind and Bakke, *H. tritici* P. Henn., *H. tritici-vulgaris* Nisikado, and *H. gramineum* Rabenh. have been recorded affecting wheat in various parts of the world. Mitra (1934) in reviewing the literature of the subject, reports *H. bicolor* Mitra and *H. halodes* Drechs var. *tritici* Mitra in wheat in India causing foot and root-rots. Since the casual comparison of the fungus under study with those affecting wheat showed distinct differences in spore form and morphology, the present study was undertaken.

Symptoms

In nature, the disease is generally found to occur on stem, leaf, leaf sheath, spike, glume and spikelet. It appears on both sides of the leaf as small brown necrotic spots, oval in shape, which gradually elongate in line with the axis of the leaf, later becoming deep brown in colour. Heavily affected leaves turn reddish-brown, droop and wither. The spots which are larger in size and deep brown in colour on leaf sheaths are not so well defined as on leaves. The spots on the stems are oblong and of light brown or sepia colour. The cortical tissue is invaded. In severe cases of infection, the portion above the spot dies off. In the diseased inflorescence, the glumes become separate and show dark brown elongated spots (Plate I, fig. 2). In severe cases of infection, the size of the grain is also affected ; the prominent symptom which is visible from a distance is the stripe-like appearence on the stems and floral axis. At times, the stalk bearing the ear may droop down.

Isolation made from affected stems yielded a pure culture of a sp. of *Helminthosporium* used in subsequent investigations.

Pathogenicity

In a preliminary experiment made using 17 day old plants, whose leaves were previously drawn between fingers to remove the bloom, were sprayed with a spore suspension and incubated in moist chambers for 48 hours showed typical necrotic areas on the 7th day whereafter there was a rapid development of the disease. The necrotic areas turning deep brown increased in size and number until, on the 10th day, the plants began to show signs of withering and blighting characteristic of the disease under humid environment. Controls remained healthy. The experiment was repeated several times and the results confirmed.

The results showed that the species of *Helminthosporium* under study is a virulent parasite causing the desease readily without wounds, although the rapidity of the development of disease depended upon humid conditions during the period of experiment.

Relation of Humidity to Development of the Disease

This was determined by first inoculating 20 day old healthy plants of Niphad 4 wheat in the usual manner and incubating them under moist chambers for varying lengths of time. The inoculated plants were then removed to the benches in the glass house and kept under observation. The results so obtained are summarised in Table I.

Table I

Relation of humidity to development of the disease in wheat

Period in hrs. in moist chambers	Number of plants inoculated	Severity of disease	Percentage Infection	Remarks
6	10	Nil	Nil	All observations were recorded on 5th day after removal from moist chamber
12	10	†	10 to 20	
24	8	††	25 to 50	
48	10	†††	40 to 60	

N. B. :—† Few sporadic spots ; †† Good number of spots restricted to leaves ; and ††† Numerous coalesced spots all over the plants and stripes on the leaves.

It will be seen from these results that the duration of the plants under humid conditions determined the severity of the disease. Forty eight hours' incubation favoured rapid development while 6 hours period inhibited the development of the disease. The results also show that the severity and the rate of development of the disease are directly proportional to the period of incubation under moist conditions.

Development of Disease in Relation to Plant Growth

Since the disease was invariably found in the heading stage in nature, it was considered worthwhile to investigate into the most critical susceptible stage of the wheat plant. For this purpose, the plants (Niphad 4) were grown in 6 inch pots, inoculated in the usual manner at fixed periods of plant growth, incubated for 48 hours and then removed to glass house benches for observations. The results show that the disease is at its maximum in fully developed plants and at the flag stage. These are in accord with the field observations made from time to time and confirm that the flag leaf stage of the host is the most critical and highly susceptible period for the development of the disease under favourable environ-

ments. The fungus is, thus, incapable of infecting young tissues and does not ordinarily produce either blights or foot rots, commonly associated with *Helminthosporium* spp.

Morphology

The following observations and descriptions of the fungus cultured on potato dextrose agar were recorded from 2 weeks old cultures grown at 30°C.

Mycelium :—The fungus produces profuse mycelial growth in Richards, potato dextrose and corn-meal agar-media. In young stage it is aerial, septate, hyaline or bluish and light brown when mature. There is a slight constriction at the septa, the average width being 6.22μ. (Branching is sparse and at an acute angle. Septation is regular except where branching is seen. Oil globules are present and their number varies from 2 to 3 in a cell). H-shaped structures between hyphae were also observed though these are not of general occurrence.

Conidia :—These are borne singly or in clusters of 1 to 9 at different points on the conidiophore, in acropetal succession. They are thick-walled, sub-cylindrical or ovate, rarely obclavate ; slightly curved and bent and in a few cases straight, wider near the centre and gradually tapering towards the ends. The end cells are hyaline, rounded off at the base giving rise to a conspicuous protruding hilum at the point of attachment. (Plate I, Fig. 3). The colour varies from dark brown to dark Otter brown ; Conidia measure 45 to 140 (mostly 62—110) $\mu \times 10$ to 16μ. The septa vary from 3 to 12.

Conidiophores :—Conidiophores are mostly erect, in most cases emerging singly, bulbous at the base with prominent geniculations, dark brown to dark Otter brown at the tip with hyaline to sub-hyaline base, with rounded hemispherical apical cell and measure 50 to 120 $\mu \times 6$ to 9μ with 3 to 5 septa. 1 to 9 conidia are borne on each conidiophore at different places. (Plate I, Fig. 5).

Germination studies

Mature conidia obtained from 8 days old culture grown on potato dextrose agar when sown in hanging drop preparations and incubated at different temperatures for five hours showed that they germinate at a wide range of temperatures varying from 5° to 42° C., the optimum being 25° to 30° C. Germination was vigorous and germ tubes longer at 25° C. (Plate I, Fig. 4); the minimum period for germination was observed to be 3 hours.

In general, each conidium gives rise to a single germ tube from one of its polar ends. The basal germ tube arises just adjacent to the hilum. In a few cases, a cell may give rise to 2 or more germ tubes. H-shaped connections were commonly observed between germ tubes of different conidia.

Growth at different temperatures

The standard medium employed for this experiment was Richards agar. Duplicate petri dishes were poured with 20 ml. of medium in each,

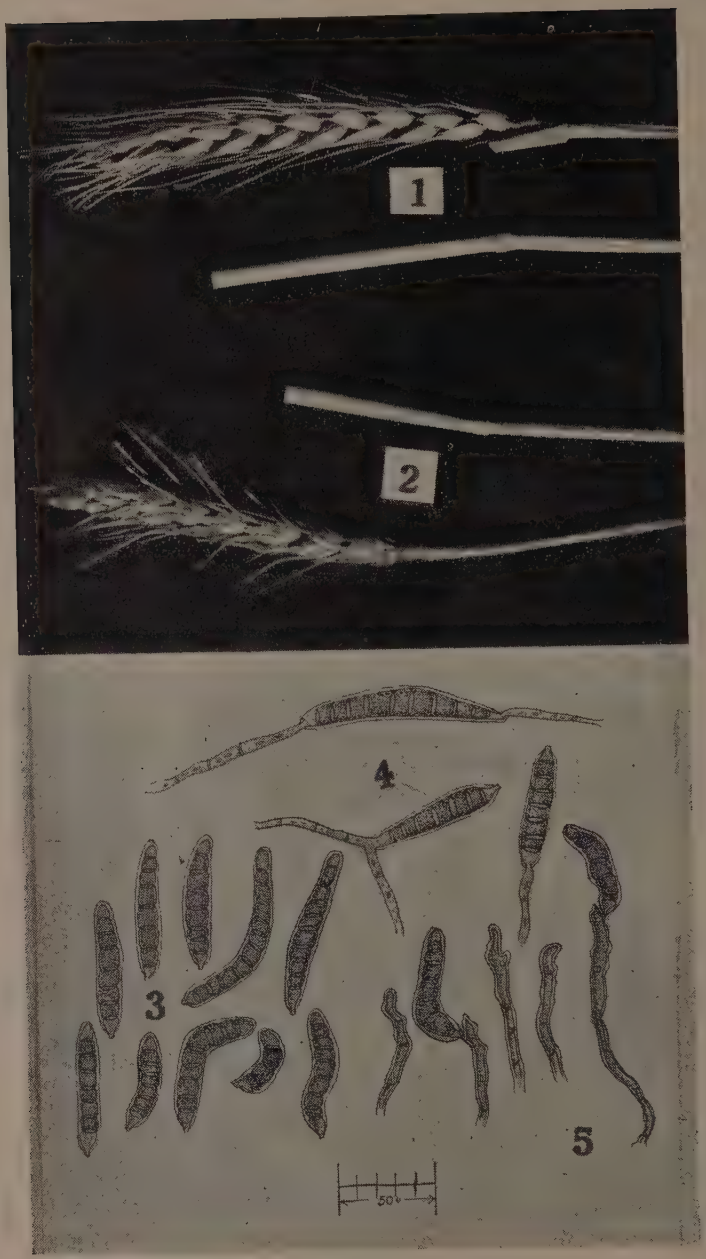

PLATE 1

Figs. 1.—Healthy earhead and stem; 2.—Affected earhead and stem (Reduced ½ natural size); 3.—Conidia; 4.—Conidia germinating; 5.—Conidiophores bearing conidia.

inoculated and kept at different temperatures. The following observations were recorded at the end of 5 days.

TABLE II

Growth of Helminthosporium sp. at different temperatures

Temperature O C.	Colony diameter in mm.	Sporulation	Growth characters
10	Nil	Nil	...
15	Trace	Nil	Whitish fuzz around the inoculum.
20	50	Moderate	Aerial growth, colour light to dark green towards centre.
25	60	Abundant	Aerial, spreading, colour light to dark green.
30	90	Abundant	Entire plate covered, colour light to dark green.
35	65	Nil	Aerial growth, colour white to orange towards centre.
40	Nil	Nil	...

The fungus produces very scanty growth at 15°C, while it was inhibited at 10° and 40°C. Although the fungus produces profuse vegetative growth at 30°C., the sporulation was best at 25°C. It appears to have a narrow growth-temperature range as compared with that for germination which was wider although the optimum is the same in both the cases. The range for sporulation lay between 20° and 30°C.

GROWTH OF HELMINTHOSPORIUM SPECIES ON DIFFERENT MEDIA

The various media were poured in uniform petri dishes in equal quantities and planted with a small amount of inoculum from a 2 week-old culture on potato dextrose agar. The petri dishes were then incubated at 30°C. and observations recorded at the end of 5 days, with regard to colony diameter, growth characters and sporulation.

It was observed that the fungus made best growth in Richards' agar, although it grew fairly well on all other media tried. Considerable variation was observed in sporulation which was at its best on Richards', gram meal, host decoction dextrose, potato dextrose and carrot agar media. Maize agar stimulated vegetative growth but suppressed sporulation, while the growth was the poorest on plain agar and sporulation absent on host decoction alone. On oat meal, lima bean, artificial potato dextrose and french bean agars, the growth and sporulation were moderate.

UTILIZATION OF CARBON COMPOUNDS

The carbon requirements of the fungus were studied at a constant temperature of 30°C. on Richards' agar (less sucrose) to which different carbon sources were added in 1 per cent concentrations.

The fungus grew well and sporulated profusely in most cases except with dulcitol, amygdalin, rhamnose, arabinose and salicin. Lactose, glucose, galactose, levulose, sucrose, raffinose, glycogen, dextrose and mannitol appeared to stimulate sporulation whereas size of conidia was considerably longer with lactose.

Utilization of Nitrogenous Compounds

The fungus appears to have a very narrow range for its nitrogen source as expressed by vegetative growth and sporulation. Potassium nitrate appeared alone to prove the best source while sodium nitrate, ammonium lactate, ammonium sulphate, ammonium tartarate, peptone, asparagin, ammonium nitrate and urea nitrate were inadequate and did not favour either good growth or sporulation. The growth was entirely inhibited in sodium nitrite and l-naphthylamine.

Enzyme Production

The comparative capacity of the fungus to produce various enzymes was studied by growing it in a standard medium as given by Crabill and Reed (1915) at a constant temperature of 30°C.

It was observed that the fungus is incapable of utilising a wide range of food materials as it makes poor growth and scanty sporulation on asparagin, gelatin, eggalbumin, casein, inulin and esculin. It secretes the enzymes cytase and emulsin in large quantities and, therefore, probably has limited parasitic capabilities.

Hydrogen-ion in Relation to Growth

The flasks containing Richards' medium adjusted to different pH values were inoculated with the fungus and incubated at 30°C for 11 days when the dry weight of the fungal mat was determined, as given in Table III below :—

Table III

Growth of Helminthosporium sp. in relation to Hydrogen-ion concentration.

Initial pH	Dry weight of mat in m. grms.
1·90	Nil
3·10	408
4·20	950
5·30	890
5·90	850
7·10	750
8·10	280
8·90	110

It will be seen from this table that the fungus grows best between pH4 and 6 but growth decreases either towards high acid or alkaline reactions. The final reactions of the medium after the fungus growth tended more towards acid side when compared colorimetrically

Over-Summering

Having regard to the nature of the fungus, there is little doubt about its ability to survive periods of droughts in stubbles, other host parts and in seed. Since, however, the fungus has a very restricted host-range, it was important to know how long it could retain its viability in culture and on the host plant.

Periodical observations were, therefore, made on the germination of conidia obtained from potato dextrose agar cultures kept at room temperature varying from 27° to 30°C. In the following table are summarised the results.

Table IV

Viability of Conidia of Helminthosporium sp. obtained from potato dextrose agar.

Date	Per cent germination
19- 9-50	93
30- 9-50	80
17-10-50	67
6-11-50	60
28-11-50	50
16-12-50	42
4- 1-51	33
23- 1-51	24

The germination counts were taken till the culture remained pure and viable. The culture dried and lost its viability after 4 months. Since the fungus in culture retained its viability for 4 months, it was thought that longer viability could be expected in infected host tissues. Accordingly, periodical isolations were made from diseased host tissues. specially obtained from floral axis and pure culture of the typical fungus obtained for a period of 10 months from the date of collection of diseased samples in the field. It is thus clear that the fungus can survive critical periods of drought in the absence of a living host.

Host Range and Varietal Resistance

Although the disease was originally observed to do extensive damage to varieties like Niphad 4 and Pusa 4 at different places, it was desirable to know the reactions of larger number of varieties grown in the State to the development of the disease. The comparative resistance of these varieties was, therefore, tested under an artificial epidemic. In addition several hybrid strains and exotic wheat varieties were also tested.

Two lots of healthy wheat plants were grown in 6 inch pots in sterilised soil and inoculated separately on 20th and 42nd day after sowing. The observations on the development of disease at the two stages and its reaction and severity were recorded at the end of 7 days after inoculation. The results are recorded in table V.

TABLE V

Varietal Resistance in Wheat to Helminthosporium sp.

S. No.	Name	Source	20 days old plants	42 days old plants
			Reaction	Reaction
1	Niphad 4 (NA)	Niphad Farm	4	4
2	Gulab	,,	4	4
3	Motiya	,,	4	4
4	Jaya	,,	4	4
5	Vijay	,,	4	4
6	Khapli	,,	4	4
7	Pusa 4	,,	4	4
8	Gabo	Maha-bleshwar	1	—
9	K. 144	,,	1	—
10	Hofed 1	,,	3	4
11	Hofed 1×N4	,,	1	2
12	K. 144×N4	,,	1	1
13	Mondhya×Hofed 1	,,	1	1
14	Kenya Governor	,,	F	1
15	Gaza	,,	4	4
16	Charter	,,	F	2
17	E. 220	,,	4	4
18	Ex. 61	,,	2	1
19	Ex. 73	,,	2	F
20	Kenya 10854	,,	4	2
21	Arnej 624	Arnej	4	2
22	Arnej 206	,,	4	3
23	53-2	Dharwar	4	4
24	120-11-1-2	,,	4	4
25	485-56	,,	4	2
26	25-28-1-1	,,	4	4
27	480-31	,,	4	1
28	N.S.-39-1	,,	4	4
29	N.S.-42	,,	4	4
30	487-58	,,	4	4
31	482-2	,,	4	1
32	179-62	,,	4	2
33	314-70	,,	4	4
34	66-1	,,	4	4
35	N.S-54	,,	4	4

N B :—4-Heavy, 3-Moderate. 2-Slight. 1-Trace. and F.-Flexing.

It is seen that most of the local types of wheat are highly susceptible to the fungus in the seedling stage while the exotic types in general show a high degree of resistance. Similarly, crosses between Indian and exotic wheats like N4 × K 144, Hofed × N4, Mondhya × Hofed, etc. show a high degree of resistance. It is of interest to note that the wheats possessing high resistance to stem rust also show a high resistance to this disease. It is also very interesting to note that *Khapli* which in nature is highly resistant to stem rust was highly susceptible to this disease in artificial inoculation experiments. Except in a few cases as in Kenya 10854, Arnej 624, 485-56, 480-31, 482-2 and 179-62, there appears to be a complete correlation between seedling and adult resistance or susceptibility.

In view of the confusion that prevails in the taxonomy of the genus *Helminthosporium* and the difficulties involved in diagnosing species purely on morphological grounds, it was very necessary to determine the exact pathogenic capabilities and host range of the fungus as well, specially in Gramineae. Experiments were, therefore, made to determine this aspect of the fungus. Twenty day old graminaceous plants of *Avena sativa*, *Eleusine coracana*, *Hordeum vulgare*, *Oryza sativa*, *Pennisetum glaucum*, *Setaria glauca*, *S. italica*, *Sorghum vulgare*, *Triticum aestivum* and *Zea mays* raised in pots were subjected to an artificial epidemic under standard humid chamber. Niphad 4 wheat was used as a control in the experiments which were repeated 3 times with similar results, which showed very clearly that the fungus is parasitic on durum and vulgare types of wheat grown in peninsular India.

The Disease

The disease in the field occurs during December-February at the heading stage of the host, when conditions are favourable. Years of heavy dew fall, late rains, irrigation and density of crop are some of the important factors that favour rapid development. Humidity plays a very important role since the development of disease coincides with periods of high humidity. This has been also corroborated in the experimental evidence presented in these pages. The wide range of temperatures for germination (5° to 42°C.) and the ability of the fungus to make its optimum growth at moderate temperature (25° to 30°C.), coupled with high humidity required for development of the disease have been fully borne out by experimental evidence. The disease made very rapid development in periods of prolonged humidity as compared with shorter periods of humid weather, which inhibited and arrested development in the inoculation experiments presented here, confirming the field observations made from time to time in respect of its phenological relationships.

Taxonomy

In view of the complicated and somewhat confused texonomy of the genus *Helminthosporium*, it was difficult to determine the exact identity of the fungus without making a close comparison with other species parasitic on *Triticum* and other graminaecous hosts. In determining the exact position and the specific rank of a fungus, the factors of spore characters and spore measurement have been found to be inadequate in view of the phenomenon of physiologic or host specialization exhibited by many fungi.

On morphological grounds, this fungus has resemblance with *Helminthosporium rostratum* Drechs., *H. halodes* Drechs., *H. halodes* var. *tritici* Mitra and *H. nodulosum* Berk & Curt. though it is distinct from these species in several material respects. It has no resemblances whatsoever with *H. tritici-repentis* Died. recorded by Mitra (1934) on wheat in India. A comparative statement showing these resemblances and differences is given below :—

The fungus	Conidial Dimensions	Septa	Graminaceous Host range
Helminthosporium rostratum	32-184 × 14-22 μ	3-15	Restricted
H. halodes	20-105 × 10-14 μ	1-12	Restricted
H. halodes var, *tritici*	23-72 × 13-20 μ	2-9	Foot rot of wheat
H. nodulosum	40-114 × 11-21 μ	3-11	Wide host range
H. tritici-repentis	45-201 × 13-22 μ	2-11	*Triticum* and *Agropyron repens*
H. specis	45-140 × 10-16 μ	3-11	Restricted to *Triticum* spp.

It will be seen that of the 5 species to which this fungus bears resemblances, the species nearest are *H. rostratum* and *H. nodulosum*. The rostrate shape of the conidia of *H. rostratum*, however, is typical of the species and therefore, is distinct from the present species. *H. tritici-repentis* has straight conidia with typical snake head and therefore does not agree with the description of the fungus under study. *H.nodulosum* on the other hand, though very close to it from the points of view of spore character and measurement has a wide host range in the graminaceous family and therefore, is distinct from the present fungus which has a restricted host range in *Triticum* spp.

In view of this highly specialized character of the fungus, it is propoposed to raise it to a varietal rank as *Helminthosporium nodulosum* var. *tritici* n. var.

Helminthosporium nodulosum var. *tritici* nov. var.

Differs from the species in having restricted host range in Triticum spp. Collected at Baramati (Poona) during February, 1948.

Helminthosporium nodulosum var. *tritici* var. nov.

Nova haec varietas differert a typica specie in eoquod varietas restringitur omnino ad species Tritici. Typus lectus in loco Baramati, Poona, monse februario anni 1948.

SUMMARY

A species of *Helminthosporium* causing stripe disease on wheat occurs frequently under conditions of moderate temperatures (25-30°C.) and prolonged high humidity.

The period of heading appears to be the most susceptible stage of the plant. In severe cases, the leaves, stem and floral axis are infected, the stem sometimes drooping at the internode.

The minimum, the optimum and the maximum temperatures for conidial germination are 5°, 25° and 42°C., respectively. They germinate readily at the end of 3 hours.

The range of temperature for growth is small viz. 20° to 35°C., the minimum between 15° and 20°C., the optimum between 25° and 30°C., and the maximum at about 35°C.

Carbon sources seem to help in profuse sporulation without any change in vegetative growth, while nitrogen sources do not seem to aid in sporulation. Acidic medium favours growth.

Local varieties of wheat in general were highly susceptible while the exotic types highly resistant. Stem rust resistant hybrids also possess high degree of resistance to this *Helminthosporium*, Khapli being otherwise. The pathogen is highly specialized and restricted. On the basis of morphology and spore characters the present fungus differs from *H. rostratum* and on host specificity from *H. nodulosum* and is, therefore assigned a varietal rank *H. nodulosum* var. *tritici* n. var.

The writers wish to express their deep gratitude to Dr. B.B. Mundkur for carefully going through the manuscript and to Mr. H.A. Dade of the Commonwealth Mycological Institute, Kew, for helpful suggestions in the identity of the fungus.

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NOTES ON SOME FUNGI FROM SOUTH INDIA II.*

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(Accepted for publication, July 4, 1953)

Homostegia derridis sp. nov.

Stromata dothideoid, black, isolated or confluent, amphigenous, clypeate, multiloculate, occupying the entire thickness of the leaf, rounded or linear, 1—3 mm. long and 1 mm. broad; loculi depressed, ostiolate, opening epiphyllously, 280—410×196—294μ; asci cylindric, 8—spored, 93×9μ (84—168×9—12), short stipitate, paraphysate; paraphyses filamentous, hyaline; ascospores fusiform, 25×5μ (19—28×3—7), 3—septate, olive brown, constricted at the septum, irregularly biseriate.

Stromata dothideoidea, nigra, separata vel confluentia, amphigena, clypeata, multiloculata, occupantia totam folii densitatem, rotundata vel linearia, 1—3 mm. longa, 1 mm. lata; loculi depressi, ostiolati, epiphylle patentes, 280—410×196—294μ; asci cylindrici, octospori, 93×9μ (84—168×9—12), brevistipitati, paraphysati, paraphysis filiformis hyalinis; ascosporae fusiformiae, 25×5μ (19—28×3—7), triseptatae, olivaceobrunneae, constrictae ad septum, irregulariter biseriatae.

On living leaves of *Derris heyneana* Benth. (*Leguminosae*), Burliar (Nilgiris), 11-4-53, N.V. Sundaram.

The stromata occur in groups as black, raised, rounded or elongated growths on both sides of the leaf. The entire thickness of the leaf is occupied by the stroma and a number of loculi are present in each stroma. The loculus extends to nearly threefourths of the thickness of the leaf and opens out by an ostiole on the upper surface. The end cells of the ascospores are tapering and may be obtuse or rarely acute. Normally the ascospores are four celled but occasionally three celled spores are also present. The spores germinate readily and the germ tube arises from a side of the end cell.

Lasiosphaeria caryophylli sp. nov.

Spots indefinite, circular or irregular, yellowish on the upper surface, thickened on the lower surface; mycelium mostly intercellular, hyaline, but becoming olive brown nearer the lower epidermis; perithecia superficial, hypophyllous, crowded, forming velvetty masses, 1—4 mm. in diameter, setose, setae dark brown, long, septate, often branched at the apex; perithecial wall of many layers; asci obovate, having a thin colourless wall, 109×37μ (93—140×34—53), 8—spored; paraphyses evanescent; ascospores arranged side by side, vermiform, 4-celled, strongly constricted at the septa, dark brown, 68×12μ (56—81×9—15).

Maculae indefinitae, circulares vel irregulares, luteolae in superiore pagina foliorum, incrassatae in inferiore pagina; mycelium ut plurimum

* The first part was published in Indian Phytopathology, 5 : 110-115, 1952

intercellulare, hyalinum, sed evadens olivaceo-brunneum ad epidermidem inferiorem; perithecia superficialia, hypophylla, acervata, efformantia massas holosericas, 1—4 mm. diam., setosa, setis fuscebrunneis, longis, septatis, saepe ramosis ad apicem; perithecii parietes pluries seriati; asci obovati, incoloro pariete praediti, $109 \times 37\mu$ ($93—140 \times 34—53$), octospori; paraphyses evanescentes; ascosporae lateraliter ordinatae, vermiformes, 4-cellulatae, fortiter constrictae ad septa, fusce brunneae, $68 \times 12\mu$ ($56—81 \times 9—15$).

On living leaves of *Syzigium caryophyllaeum* Gaertn. (Myrtaceae), Kasaragod taluk (South Kanara), 25-2-53. T. S. Ramakrishnan.

The infection spots are visible on the upper surface as yellow indefinite areas which later on change into brown. On the lower surface are prominent black cushiony and velvetty growths made up of clusters of perithecia. The perithecium is entirely superficial and is provided with a dense covering of dark brown setae, many of which are branched repeatedly at the apex and tapering. The terminal cells of these branches are lighter coloured. The mesophyll of the leaf is thoroughly invaded by the mycelium consisting of branched hyaline hyphae. Aggregates of hyphal cells are noticed in the substomatal air spaces. These clusters assume an olive brown colour and form the connecting links with the external growths of the fungus. The perithecium has a multilayered wall the outer layers of which are thick walled and dark brown. No definite ostiole is recognisable. The setae originate from the outer layers of the perithecia and form dense growths. The asci are broad with thin walls. The ascospores are typically 4-celled and cylindrical with constriction at the septa. In the two end cells one or more subhyaline transverse zones are visible simulating caps. The paraphyses are slender but very soon gelatinise.

Phaeodothis cordifoliae sp. nov.

Spots yellowish brown, irregular, amphigenous, stromata clustered or single in each spot, black, shiny, amphigenous with minute projections on the upper surface, clypeate, occupying the upper half of the mesophyll, multiloculate; loculi $336—742 \times 280—420\ \mu$, depressed, ostiolate; asci very much elongated, 8-spored, wall hyaline, paraphysate; paraphyses distinguishable in the immature stage; ascospores 8, irregularly arranged in the ascus, oblong, rounded ends, two celled, constricted at the septum, brown in colour, $22 \times 16\ \mu$ ($19—28 \times 12—19$).

Maculae luteo-brunneae, irregulares, amphigenae; stromata aggregata vel singula, nigra, micantia, amphigena, minutis projectionious in superiore pagina ornata, clypeata, occupantia mediam partem superiorem mesophylli, multiloculata; loculi $336—742 \times 280—420\ \mu$, depressi, ostiolati; asci valde elongati, paraphysati, paraphyses distinctae in immatura conditione; ascopsorae 8, irregulariter dispositae in asco, oblongae, apicibus rotundis, bicellulatae, constrictae ad septa, brunneae colore, $22 \times 16\mu$ ($19—28 \times 12—19$).

On living leaves of *Jasminum cordifolium* Wall. (Oleaceae), Burliar (Nilgiris), 11-4-53, N. V. Sundaram.

The stromata are well developed and extend into the mesophyll. On the surface they appear as elevated black shining bodies. Asci are distinguishable only when young. When mature they get disorganised and all the spores collect in groups embedded in the gelatinous substance inside the loculus.

Phyllachora kanarensis Sp. nov.

Infection spots irregular, black, shining, amphigenous; stromata 2—9 mm. irregular in shape, clypeate showing on both sides of the leaf, locules many, showing externally as raised points, subglobose, ostiolate, 168—340×126—288 μ; asci long, cylindric, stipitate, hyaline, 87×9μ (69—99×7·5—11), 8-spored, paraphyses numerous, hyaline; ascospores monostichous, oblong, hyaline, 12×6μ (9—15×4—7·5).

Infectionis maculae irregulares, nigrae,nitentes, amphigenae; stromata 2—9 mm. diam., irregularia forma, clypeata, visibilia in utraque pagina foliorum, loculis plurimis externe visibilibus ut punctis elevatis, subglobosa, ostiolata, 168—340×126—288 μ; asci longi, cylindrici, stipitati, hyalini, 87×9 μ (69—99×7·5—11), octospori; paraphyses plurimae, hyalinae; ascosporae monostichous, oblongae, hyalinae, 12×6 μ(9—15×4—7·5)

On living leaves of *Hopea wightiana* W. (Dipterocarpaceae) Mangalore (South Kanara), 25-2-53, T. S. Ramakrishnan.

This fungus is very common throughout the year.

Phyllachora scolopiae sp. nov.

Spots circular, black with a brown halo, shining, amphigenous, 2—7 mm. in diameter with punctiform elevations on both sides; clypeus well developed on both sides, stroma occupying the entire thickness of the leaf, stromata multilocular; loculi ostiolate, ovate or flattened, 140—240 ×154—204 μ; asci cylindric-clavate shortly stipitate, hyaline, 8-spored, 84×12 μ (53—108×9—15), paraphysate, paraphyses hyaline, filamentous, ultimately gelatinising; ascospores obliquely arranged, one celled, spindle shaped, hyaline, 24×4·5 μ (15—28×3—6).

Maculae circulares, nigrae, ornatae nimbo brunneo, nitentes, amphigenae, 2—7 mm. diam., punctiformiter elevatae in utraque pagina; clypeus bene evolutus in utroque latere, stroma implens totam densitatem folii, multiloculare; loculi ostiolati ovati vel applanati, 140—240×154—204 μ; asci cylindriciclavati, breviter stipitati, hyalini, octospori, 84×12μ (53—108×9—15), paraphysati, paraphysibus hyalinis, filamentosis, tandem gelatinosis; ascosporae oblique dispositae, semel cellulatae, fusiformes, hyalinae, 24×4·5 μ (15—28×3—6).

On living leaves of *Scolopia crenata* Clos. (Bixaceae), Burliar (Nilgiris), 22-3-53, T.S. Ramakrishnan and N.V. Sundaram.

The mycelium between the two clypei forms a close network of inter and intracellular, septate, hyaline or olivaceous brown hyphae. The locules which are many in each stroma are usually arranged in one series occupying five-sixth of the thickness of the leaf. They open on one or the other of the surfaces through narrow ostioles crowded with numerous hyaline *periphyses*. Associated with each stroma is what is apparently the

conidial stage consisting of subepidermal erumpent acervuli arranged along the margin of the stroma. Conidia are hyaline, one celled, oblong and measure $4.8 \times 3.6\mu$ ($4.2-6 \times 2.5-4$). They are produced on hyaline, closely packed elongated conidiophores.

Aecidium iquitosense P. Henn.

Saccardo, P.A.. *Syll. Fung.*, *17*, 415, 1905.

On leaves of *Psychotria elongata* Hk. (Rubiaceae), Burliar (Nilgiris), 11-4-53, N.V. Sundaram.

Yellowish hypertrophied spots are formed on the leaves and veins. The aecia are hypophyllous and cupulate. One to eight aecia may be formed in a spot. The pycnia are hypophyllous, prominent, oval or elliptic, deeply sunk, ostiole being flush with the epidermis ; the contents are orange coloured. The aeciospores are sub-globose to angular with verrucose wall. Three species of *Aecidium* have been recorded on the host genus. But from the characters of the rust under study it has been identified as above.

Puccinia brizae-maximae sp. nov.

Uredia amphigenous, elongated, brown, sub-epidermal, erumpent ; urediospores on long stalks, oblong to obovate, $31 \times 19\ \mu$($25-40 \times 15-22$), wall coloured light brown, verrucose, with four equatorial germpores ; telia black, elongated, on the leaf-sheath and stem, sub-epidermal, erumpent ; teliospores oblong, rounded at the apex, slightly tapering towards the base, $35 \times 19\ \mu$($25-47 \times 15-25$), apex thickened up to $12.4\ \mu$, orange-cinnamon coloured, pedicellate with long, brown pedicel, more than $40\ \mu$ in length ; mesospores mixed with teliospores, elliptic, $22-37 \times 12-16\ \mu$, apex thickened.

Uredia amphigena, elongata, brunnea, subepidermalia, erumpentia ; uredosporae longis pedunculis insidentes, oblongae ad obovatae, $31 \times 19\ \mu$ ($25-40 \times 15-22$), parietibus coloratis pallide brunneis, verrucosis, quattuor germinationis poris ornatae ; telia nigra, elongata, foliorum-vaginae atque culmis insidentia, subepidermalia, erumpentia ; teliosporae oblongae, ad apicem rotundatae, tenuiter fastigatae ad basim, $35 \times 19\ \mu$($25-47 \times 15-25$), apice incrassato ad $12.4\ \mu$, citreocinnamomeo, pedicellatae longo, brunneoque pediculo, plus $40\ \mu$ longo ; mesosporae mixtae teliosporis, ellipticae, $22-37 \times 12-16\ \mu$, apice crasso.

On *Briza maxima* L. (Gramineae), Agricultural Research Station, Nanjanad, 14-3-1953, T.S. Ramakrishnan.

Puccinia graminis has been recorded on this host. The rust under study has resemblance to *P. graminis* but the presence of mesospores which have not been recorded in *P. graminis* indicate that this rust is different. Further, inoculations with the urediospores on wheat, barley, oats and rye gave negative results. For these reasons it is considered to be a new species.

Puccinia garnotii sp. nov.

Uredia scattered or crowded, hypophyllous, subepidermal, erumpent, $0.25-0.75 \times 0.25$ mm. ; urediospores pedicellate, obovate, verrucose, wall

coloured, uniformly thick, 36×22 μ ($25-46 \times 19-28$) with 4-6 scattered germpores ; telia hypophyllous, similar to uredia, dark brown, subepidermal, erumpent ; teliospores two celled, clavate, lower cell longer, tapering towards the base, upper cell shorter, flattened or obtuse at the apex, wall thin, sometimes slightly thickened at the apex, prominently constricted at the septum, 46×22 μ ($40-80 \times 16-22$).

Uredia dispersa vel aggregate, hypophylla, subepidermalia, erumpentia, $0.25-0.75 \times 0.25$ mm., uredosporae pedicellatae, obovatae, verrucosae, parietibus coloratis, uniformiter crassis, 36×22 μ ($25-46 \times 19-28$), 4-6 germinationis poris dispersis ornatae ; telia hypophylla, urediis similia, fuscebrunnea, subepidermalia, erumpentia ; teliosporae 2-cellulatae, clavatae, cellula inferiori longiori, fastigata ad basim, superiori vero breviori, complanata vel obtusa ad apicem, pariete tenui praeditae, nonnumquam tenuiter solidatae ad apicem, prominenter constrictae ad septum, 46×22 μ ($40-80 \times 16-22$).

On living leaves of *Garnotia arundinacea* Hk. (Gramineae), Burliar (Nilgiris), 22-3-52, T.S. Ramakrishnan and N.V. Sundaram.

This rust forms crowded sori on the lower surface of the leaf-blade. The telia are characteristic with closely arranged teliospores. These spores have very short stalks up to 22 μ and the colour of the wall gradually deepens from the base to the apex, the latter being dark brown. This rust differs from the other species recorded on the allied host plants and is therefore described as new.

Puccinia pogonatheri Petch

Saccardo, P.A., *Syll. Fung.*, *23*, 743, 1925.

On living leaves of *Pogonatherum paniceum* Hack. (Gramineae), Singampatti (Tirunelveli), 2-10-1952, N.V. Sundaram.

Both the uredia and telia are mixed together and often teliospores are formed in older uredia. The paraphyses are distributed throughout the sorus and vary in colour from light to dark brown. The teliospores are markedly thickened at the apex. The upper cell is more deeply coloured than the lower cell. Mesospores are also found mixed with the teliospores. These are thickened at the apex and measure $22-34 \times 22-28$ μ.

Uredo brachylepissae sp. nov.

Uredia hypophyllous, in scattered clusters, subepidermal, erumpent, bright orange, powdery, aparaphysate ; urediospores oblong to obovate, 25×22 μ ($22-34 \times 19-25$), pedicellate, strongly aculeate over most of the surface, irregularly thickened, contents bright orange, with 3-4 germpores below the middle.

Uredia hypophyllae, acervulis dispersis, subepidermalia, erumpentia, clare aurantiaca, pulverulenta, aparaphysata; uredosporae oblongae vel ovatae, $25 \times 22\mu$ ($22-34 \times 19-25$) pedicellatae, distincte aculeata per totam superficiem, irregulariter crassae, 3—4 germinationis poris ornatae ad medium, quae in eis continentur lucide aurantiaca.

On living leaves of *Brachylepis nervosa* W. & A. (Asclepiadaceae) Burliar (Nilgiris), 11-4-53, N.V. Sundaram.

The uredia occur hypophyllously isolated or in small clusters. They are very prominent because of their bright orange colour. The variation in size is very wide. The urediospores are usually narrowed towards the base and on one side the wall is completely smooth while the rest of the surface is provided with prominent spines.

Uredo celastri-paniculatae sp. nov.

Uredia hypophyllous, minute, scattered or in groups, subepidermal, erumpent, pulverulent; urediospores subglobose to pyriform, $28 \times 22\mu$ ($22-34 \times 19-25$), bright orange when young, strongly aculeate, with marginal, clavate, thin walled paraphyses, germpores about four in number, arranged along the middle.

Uredia hypophylla, minuta, dispersa vel aggregata, subepidermalia, erumpentia, pulverulenta; uredosporae subglobosae vel pyriformes, $28 \times 22\mu$ ($22-34 \times 19-25$), lucide aurantiacae in prima aetate, fortiter aculeatae, ornatae paraphysibus marginalibus, clavatis atque tenuiter parietatis; germinationis pori ca. 4, dispositi ad medium.

On living leaves of *Celastrus paniculata* Willd. (Celastraceae), Burliar (Nilgiris), 11-4.53, N. V. Sundaram.

The rust produces numerous sori on the lower surface of the leaf having an orange brown colour. As the sori become older the orange colour gives way to brown.

Pucciniastrum celastri Syd. has been recorded on this host genus from India but the rust under study does not possess any peridial covering for the uredia and is therefore considered to be different.

Uredo pileae Barclay

Saccardo P. A., *Syll. Fung.*, *11*, 227,1905.

On living leaves of *Pilea trinervia* W. (Urticaceae), Burliar (Nilgiris), 11-4-53, N. V. Sundaram.

The uredia are mostly hypophyllous subepidermal and erumpent. The urediospores are pedicellate, obovate or oblong with strongly verrucose wall and orange coloured contents.

Uredo rhinacanthi sp. nov.

Rust spots amphigenous, minute, circular to irregular, grey on the upper side; uredia minute, 0.1—0.2 mm., arranged in rings or scattered, subepidermal, erumpent, light brown; urediospores subglobose to oval, $27 \times 22\mu$ ($22-34 \times 19-25$), thin walled, subhyaline to light brown, verrucose, pedicellate, pedical short, paraphysate, paraphyses few, clavate, scattered.

Maculae amphigenae, minutae, circulares vel irregulares, griseae in superiore pagina; uredia minuta, 0.1—0.2 mm., disposita in annulos vel

dispersa, subepidermalia, erumpentia, pallide brunnea; uredosporae subglobosae vel ovatae, $27 \times 22\mu$ ($22-34 \times 19-25$), tenuibus parietibus praeditae, subhyalinae vel pallide brunneae, verrucosae, pedicellatae, pediculo brevi; paraphyses rarae, clavatae, dispersae.

On living leaves of *Rhinacanthus communis* Nees. (Acanthaceae), Burliar (Nilgiris), 11-4-53, N. V. Sundaram.

The incidence of the rust is evident by the numerous minute sori on the lower surface of the leaf. Sometimes these sori develop in rings round a central sorus. In such cases the infection spots are visible on the upper surface of the leaf also as circular greyish spots. The sorus develops underneath the epidermis. Sticking to the lower surface of the epidermis is a layer of fungal cells forming a roof like covering (resembling a peridium). Along the margin are several layers of closely packed vertically arranged hyphal cells resembling a palisade tissue. A few thin walled clavate, straight or bent paraphyses are found in some sori. These may be found in any part of the sorus.

Uredo ravennae Maire.

Saccardo, P. A., *Syll. Fung.*, *23*, 931, 1925.

On living leaves of *Erianthus arundinaceus* (Retz.) Fesw. (Gramineae), Coimbatore, 10-12-52, N. V. Sundaram.

The rust spots are yellowish brown. Uredia occur on both sides of the leaves as narrow, elongated sori arranged in lines. Paraphyses are absent. Urediospores have bright orange contents. The wall is either uniformly thin or sometimes thickened at the apex and sparsely echinulate. Three to five equatorial germpores are present. The spores measure $40 \times 28\mu$ ($25-53 \times 18-31$). This rust bears a close resemblance to *U. ravennae* and is identified as such.

Uredo emiliae-scabrae sp. nov.

Uredia amphigenous, dark brown, erumpent, pulvinate, distributed all over the blade and petioles; urediospores sub-globose, brown, wall coloured, of uniform thickness, $21 \times 19\mu$ ($19-24 \times 16-22$), 3—4 germpores, paraphyses absent.

Uredia amphigena, fusce brunnea, erumpentia, pulvinata, dispersa per totam laminam atque petiolum; uredospores subglobosae, brunneae, parietibus coloratis, uniformiter solidatis, $21 \times 19\mu$ ($19-24 \times 16-22$), ornatae 3—4 germinationis poris, paraphyses nullae.

On living leaves of *Emilia scabra* DC. (Compositae), Burliar (Nilgiris) 22-3-53, T. S. Ramakrishnan and N. V. Sundaram.

U. emiliae-zeylanicae Syd. (Saccardo 1930) has been recorded on *E. zeylanica* from Ceylon but the rust under study differs from that in the absence of paraphyses and in size of the urediospores.

Uredo uguressae Petch

Petch, T., *Ann. Roy. Bot. Gardn. Peradeniya*, *4*, 303, 1909.

On living leaves of *Scolopia crenata* Clos. (Bixaceae), Burliar (Nilgiris), 22-3-53, T. S. Ramakrishnan and N. V. Sundaram.

This rust produces one or more circular brown spots on the leaves. Uredia alone are present hypophyllously. One or two sori occupy the centre of the spot. Sorrounding this concentric circles of isolated or confluent uredia develop. Older uredia are brown while younger ones are bright yellowish orange. The urediospores are obovate, subglobose or pyriform, aculeate over the upper portion but smooth towards the base. Closely arranged paraphyses are present in the margin as well as in other parts of the sorus. These paraphyses are pointed or incurved and one or two celled. The spores measure 22×19 μ (15—28×15—22). Telia were not observed.

H. & P. Sydow (1914) have described *U. scolopiae* on the same host from Japan which has a great resemblance to the rust under study except that paraphyses have not been mentioned in the description of the former.

Stilbospora celtidis sp. nov.

Spots minute, hypophyllous, brown, more or less round, up to 0.2 mm. in diameter ; acervulus usually one in the centre of each spot producing black, slender, straight or curved sporehorn, up to 0.5 mm. in length, hypophyllous, subepidermal ; conidia light olivaceous, 2-4 septate, 32×3 μ (19—44×2.5—4.5), straight or flexuose, sometimes slightly constricted at the septa.

Maculae minutae, hypophyllae, brunneae, plus minusve rotundae, usque ad 0.2 mm. diam. ; acervuli ut plurimum singuli in maculis singulis, producentes sporarum cornu, nigrusm tenue, rectum vel curvatum, usque ad 0.5 mm. longum, hypophylli. subepidermales ; conidia pallide olivacea, 2-4-septata, 32×3 μ (19—44×2.5—4.5), recta vel flexuosa, nonnumquam tenuiter constricta ad septa.

On living leaves of *Celtis tetrandra* Roxb. (Ulmaceae), Burliar (Nilgiris), 11-4-53, N.V. Sundaram.

Titaeospora daphniphylli sp. nov.

Spots amphigenous, irregular, brown, acervuli amphigenous, predominently epiphyllous, black, punctate, sub-epidermal, stromata pseudoparenchymatous, dark brown, giving rise to numerous continuous light-olive-brown conidiophores from the upper portion ; conidiophores non-septate, flexuous, 24—40×3—6 μ ; conidia filiform, variously bent, sub hyaline, septa indistinct, 37×3 μ (22—62×2—4.5).

Maculae amphigenae, irregulares, brunneae ; acervuli amphigeni, ut plurimum epiphylli, nigri, punctati, subepidermales ; stromata pseudoparanchymatica, fusce brunnea, in superiore parts producentia plures conidiophoros continuous, pallide olivaceobrunneos ; conidiophori haud septati, flexuosi, 24—45×3—6 μ, conidia filiformia, varie curvata, subhyalina, septis indistinctis, 37×3 μ (22—62×2—4.5).

On living leaves of *Daphniphyllum neilgherrense* Ros. (Euphorbiaceae), Burliar (Nilgiris), 11-4-53, N.V. Sundaram.

The spots are of various shapes and bear numerous acervuli as black dots on the upper surface. Very rarely these are seen on the lower surface also. The stromata are dark coloured and well developed having a sclerotioid appearance before the formation of conidiophores. They are

deep seated and burst through the epidermis. The conidiophores are formed on the exposed surface. The conidia are never straight and are broader nearer the base and tapering towards the apex. Partition walls are not very clear.

We are indebted to Rev. Dr. H. Santhapau for the latin translation. The Curator, Royal Botanic Gardens, Calcutta and the Systematic Botanist and Professor of Botany, Coimbatore helped us in the identification of some of the host plants and we are grateful to them for their help.

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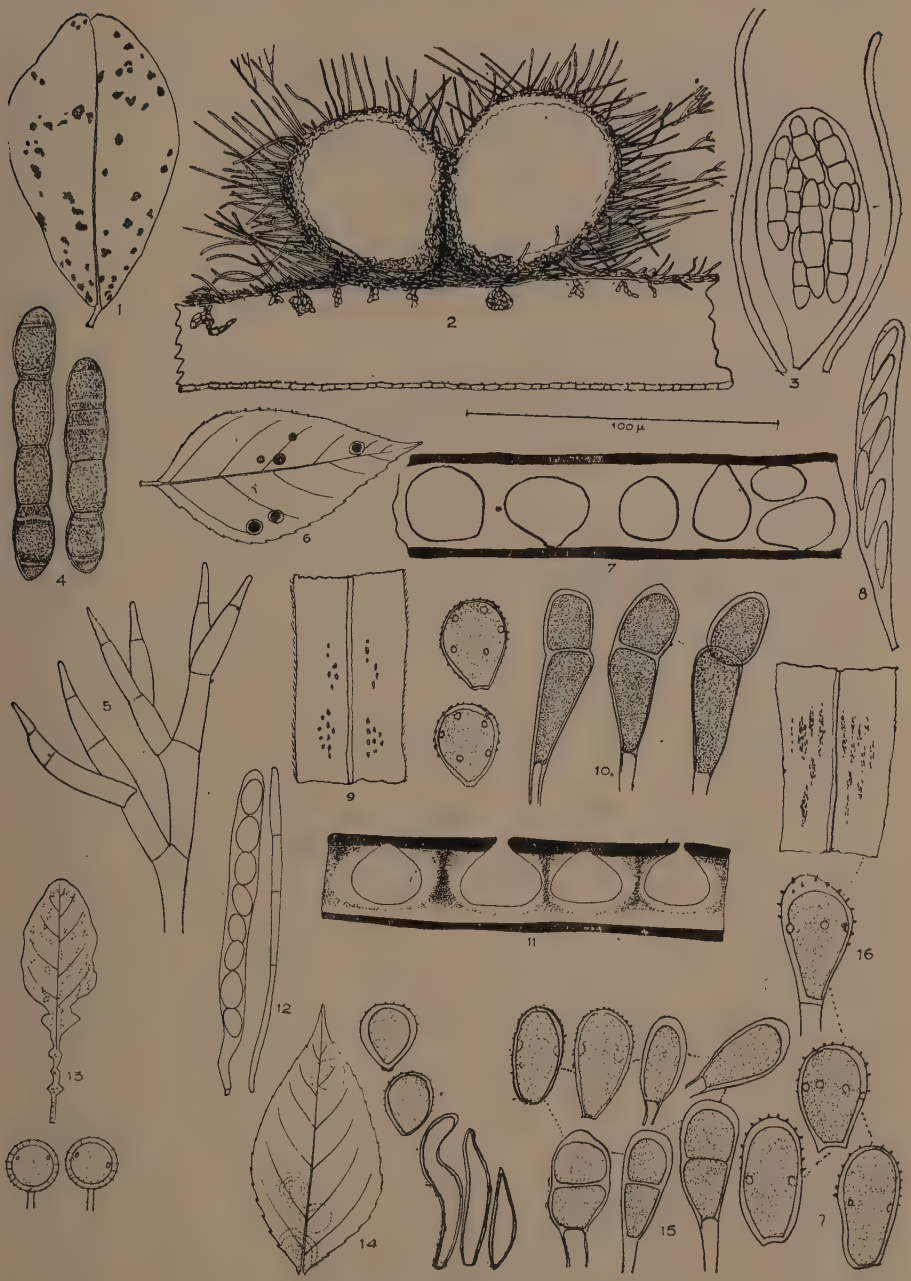

PLATE I

Notes on some Fungi from South India—II

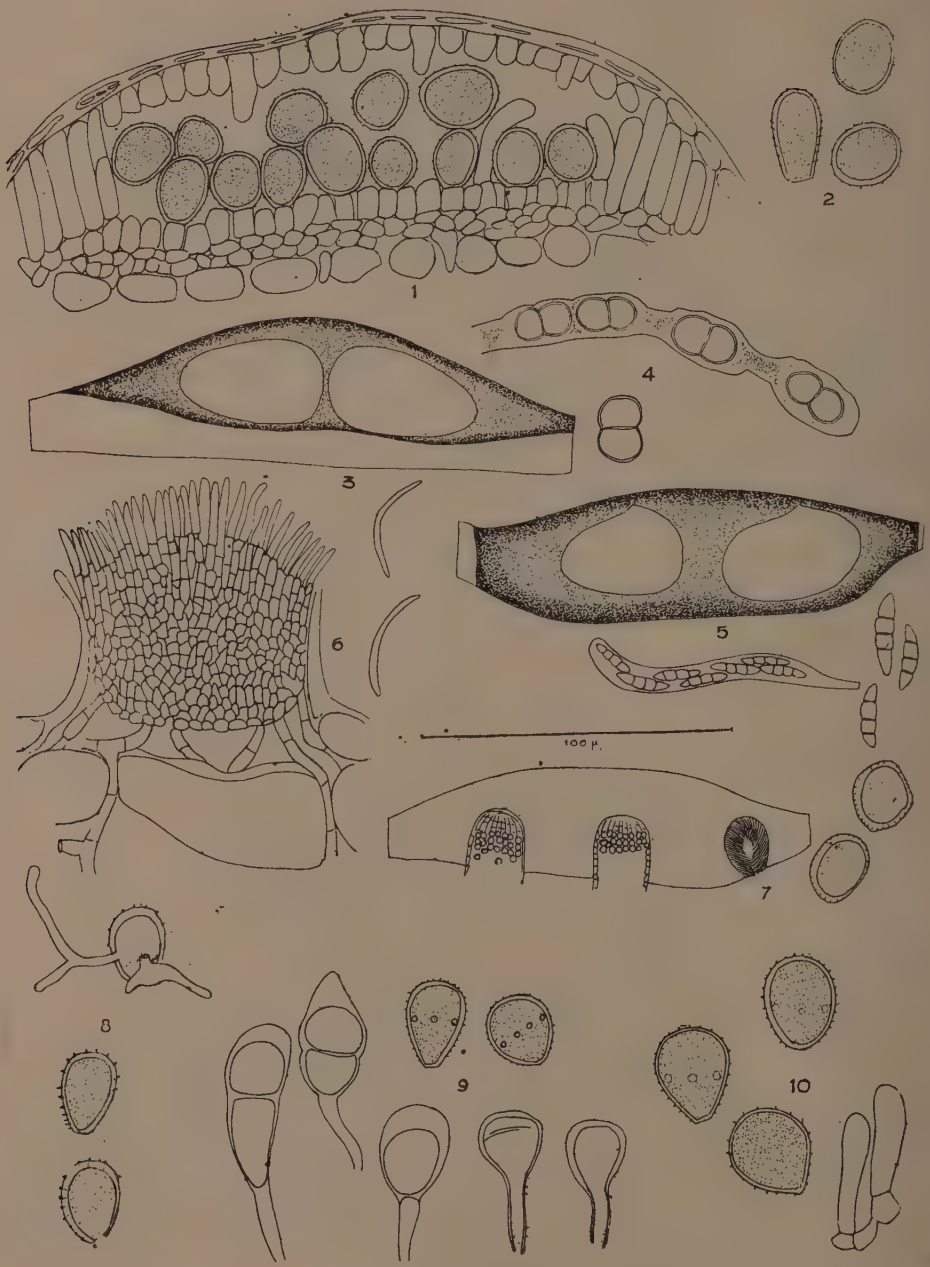

PLATE II

Notes on some Fungi from South India.—II

SUSCEPTIBILITY OF SOME GRASSES TO CEREAL RUSTS

R. S. Vasudeva, L. M. Joshi and V. C. Lele

(Accepted for publication, September 17, 1953)

Grasses are known to be collateral hosts of cereal rusts. Some grasses have been observed to be susceptible in nature whereas others have been shown to be susceptible when artificially inoculated with rusts under glass house conditions. Sufficient information on the subject in India, is not however, available. In the present investigation reactions of certain grasses, most of which are exotics, to black, brown and yellow rusts of wheat and black rust of oat were studied. The tests were particularly directed to find out if some of the exotic grasses, imported in recent years are susceptible and could possibly serve as collateral hosts to these rusts if they get naturalised in this country.

Butler (1918), and Butler and Bisby (1931) recorded the occurrence of *Puccinia graminis* Pers. on *Festuca gigantea*, *F. kashmiriana* and *Brachypodium sylvaticum*. Butler (1918) also recorded *Puccinia glumarum* (Schm.) Erikss. and Henn. on *Phalaris minor* and *Brachypodium sylvaticum*. In 1940 Mehta demonstrated by inoculation experiments the presence of *P. graminis tritici* on *Bromus patulus*, *Brachypodium sylvaticum* and *Avena fatua* growing in Simla Hills. Prasada (1948) found that the black rust of wheat could infect *Agropyron longearistatum*, *A. semicostatum*, *A. repens*, *Festuca gigentea* and *F. myuros* whereas *Puccinia glumarum* from wheat could infect *Agropyron longearistatum*, *A. semicostatum*, *Aegilops caudata* and *Bromus patulus*. The black stem rust of oat could successfully be transferred to *Bromus patulus*, *Avena fatua* and few other grasses. He also recorded occurrence of *P. graminis tritici* on 12 exotic grasses in nature (Prasada, 1951).

Experimental :

In the first instance grasses which have been reported to be susceptible to wheat rusts, such as the species of *Aegilops*, *Agropyron*, *Bromus*, *Lolium* and *Hordeum* were taken up for infectivity tests. *Phalaris* and *Avena* species were also included as black rust of oat (*Puccinia graminis avenae*) is known to infect these. All the grasses under test were from imported collection but some of them which are listed below are reported to occur in the hills of India :

Lolium rigidum, *L. temulentum*, *Bromus mollis*, *B. catharticus*, *Phalaris minor*, *Avena fatua* and *Hordeum murinum*.

The testing was done separately against mixture of races of *Puccinia graminis tritici*, *P. graminis avenae*, *P. glumarum* and *P. triticina*. Each race was first raised on Agra Local wheat, an indigenous susceptible variety of *Triticum vulgare*, and later all the races of one rust were mixed together, as far as possible, in equal quantity. Races of oat rust were raised on local oat first and then mixed together. The following races, inoculum

of which was obtained from single spore cultures, maintained at Simla Sub-station were used for inoculation purposes.

(*i*) *Puccinia graminis tritici* :—Races 15, 21, 24, 34, 40, 42, 75, 117 and 194.

(*ii*) *P. graminis avenae* :—Races 3, 4, 6 and 7.

(*iii*) *P. triticina* :—Races 10, 11, 20, 26, 63, 106, 107 and 108.

(*iv*) *P. glumarum* :—Races 13, 19, 20, 31, A*, D*, E*, F*, G* and H*.

The grasses were sown in 6″ pots and the first leaf, when it had fully unfolded, was inoculated by applying the inoculum with a lancet needle and spraying the suspension of spores with an atomiser. The inoculated pots were kept in the inoculation chamber for 48 hours and then transferred to benches in the glasshouse. The work was carried out in a spore-proof glasshouse and all necessary precautions to avoid contamination were taken. Recording was done 8—10 days after incubation period.

Twenty nine grasses were inoculated in the seedling stage separately against mixtures of races of black, brown and yellow rusts of wheat and black rust of oat. The results of these tests are summarised in the next table.

From the table it will be seen that *Agropyron pauciflorum*, *Aegilops triuncialis*, *Hordeum distichon*, *Bromus mollis*, *B. japonicus* and *Avena fatua* were heavily or moderately infected by *P. g. tritici* whereas *Agropyron scabrum*, *Hordeum stenostachys* and *H. murinum* showed only light infection and *Agropyron elongatum*, *A. sibiricum*, *Bromus coloratus* and *B. catharticus* got infected weakly producing resistant type of pustules. With *Puccinia triticina* only two grasses namely *Aegilops triuncialis* and *Hordeum distichon* showed moderate infection and *B. mollis* was found to be lightly susceptible ; *Agropyron desertorum* and *A. sibiricum* were infected very weakly. The remaining species were either immune or highly resistant. Tests with yellow rust, *P. glumarum* showed that a fairly large number of grasses are susceptible to it. Ten grasses, viz. *Agropyron elongatum*, *A. obtusiusculum*, *A. scabrum*, *A. sibiricum*, *A. desertorum*. *A. pauciflorum*, *Aegilops triuncialis*, *Hordeum stenostachys*, *Bromus catharticus* and *B. japonicus*, were heavily infected whereas *Hordeum distichon* showed light infection.

Black rust of oat, *P. graminis avenae* infected many grasses but chiefly species of *Avena* and *Phalaris*. *Phalaris paradoxa*, *P. platensis*, *Avena fatua*, *A. strigosa*, *Bromus mollis* and *B. japonicus* were heavily or moderately infected but *Phalaris minor*, *P. brachystachys*, *P. angusta*, *P. tuberosa*, *Avena ludoviciana* and *Agrophyron pauciflorum* were only lightly infected. *Lolium rigidum* and *Phalaris canariensis* were, however, weakly infected. The other grasses tested were mostly immune but a few showed high degree of resistance.

Bromus catharticus, *B. japonicus* and *Hordeum murinum* were also found infected in nature by yellow rust of wheat at the Indian Agricultural Research Institute, New Delhi. *B. catharticus*, has also been reported from South India to be a collateral host of yellow rust. The presence of

Note :—Races marked with asterisks (*) are new and have not been assigned the numbers in the International keys.

Reactions of 29 grasses to black, brown and yellow rusts of wheat and black rust of oats.

Serial No.	Name	Puccinia graminis tritici			Puccinia triticina			Puccinia glumarum			Puccina graminis avenae		
		No. of trials	No. of seedlings *infected* / *inoculated*	Reactions	No. of trials	No. of seedlings *infected* / *inoculated*	Reactions	No. of trials	No. of seedlings *infected* / *inoculated*	Reactions	No. of trials	No. of seedlings *infected* / *inoculated*	Reactions
1	*Agropyron elongatum*	2	1/19	R Weak	2	0/20	R	2	17/17	S Heavy	2	0/17	I
2	*Agropyron obtusiusculum*	2	0/16	R	1	0/5	R	1	3/3	S Heavy	1	0/5	R
3	*Agropyron scabrum*	2	6/11	SR Light	2	0/11	R	1	5/5	S Heavy	1	0/6	R
4	*Agropyron sibiricum*	3	1/20	R Weak	2	2/17	R Weak	2	12/12	S Heavy	2	0/16	I
5	*Agropyron desertorum*	2	0/19	R	2	1/13	R Weak	2	18/18	S Heavy	2	0/21	I
6	*Agropyron pauciflorum*	2	24/26	S Moderate	2	0/22	R	2	15/15	S Heavy	3	28/38	SR Light
7	*Aegilops triuncialis*	2	21/21	S Moderate	2	17/17	S Moderate	2	20/20	S Heavy	2	0/19	I
8	*Lolium multiflorum*	2	0/39	I	2	0/21	I	2	0/18	R	3	0/46	R
9	*Lolium rigidum*	2	0/36	I	3	0/57	I	2	0/20	R	2	7/40	SR Weak

Reactions of 29 grasses to black, brown and yellow rusts of wheat and black rusts of cats.—(Contd.)

Serial No.	Name	Puccinia graminis tritici			Puccinia triticina			Puccinia glumarum			Puccinia graminis avenae		
		No. of trials	No. of seedlings *infected* / *inoculated*	Reactions	No. of trials	No. of seedlings *infected* / *inoculated*	Reactions	No. of trials	No. of seedlings *infected* / *inoculated*	Reactions	No. of trials	No. of seedlings *infected* / *inoculated*	Reactions
10	*Lolium remotum*	3	0/42	I	3	0/47	I	2	0/22	R	2	0/34	I
11	*Lolium persicum*	3	0/56	I	2	0/40	I	2	0/18	R	2	0/41	I
12	*Lolium temulentum*	2	0/28	R	3	0/63	I	2	0/16	R	2	0/31	I
13	*Hordeum stenostachys*	3	42/47	SR Light	2	0/25	I	2	13/13	S Heavy	2	0/38	I
14	*Hordeum murinum*	3	14/18	SR Light	3	0/20	R	2	—	—	2	0/14	R
15	*Hordeum distichon*	3	37/37	S Heavy	2	34/34	S Moderate	2	20/20	SR Light	2	0/38	I
16	*Bromus mollis*	3	51/51	S Heavy	2	33/40	SR Light	2	0/20	R	3	45/45	S Heavy
17	*Bromus coloratus*	2	30/32	R Weak	3	0/43	R	2	—	—	3	0/48	R
18	*Bromus catharticus*	3	40/52	R Weak	2	0/39	R	2	21/21	S Heavy	3	0/50	R

19	*Bromus japonicus*	3	46/46	S Heavy	2	0/43	R	2	19/19	S Heavy	2	35/35	S Heavy
20	*Phalaris brachystachys*	2	0/24	I	2	0/37	I	2	0/9	R	3	5/43	SR Light
21	*Phalaris canariensis*	2	0/28	R	3	0/45	R	2	0/16	R	2	15/28	R Weak
22	*Phalaris platensis*	2	0/39	I	2	0/44	I	2	0/14	R	2	20/36	S Moderate
23	*Phalaris angusta*	2	0/40	I	2	0/41	I	2	0/20	R	2	18/39	SR Light
24	*Phalaris minor*	2	0/41	R	3	0/53	I	2	0/25	R	2	40/42	SR Light
25	*Phalaris paradexa*	2	0/42	R	2	0/36	I	2	0/15	R	3	63/63	S Moderate
26	*Phalaris tuberosa*	2	0/43	R	2	0/43	R	2	0/27	R	3	53/53	SR Light
27	*Avena ludoviciana*	2	0/31	R	2	0/30	I	2	0/21	R	2	42/42	SR Light
28	*Avena fatua*	2	19/19	S Moderate	2	0/32	I	2	0/16	R	2	39/39	S Moderate
29	*Avena strigosa*	3	0/48	R	2	0/27	I	2	0/16	R	3	52/52	S Moderate

I = Immune
R = Flecking or drying of leaf tips. Pustules usually absent; if present very small and surrounded by necrotic zones.
S = Pustules big, without necrotic zones.
SR = Both types of pustules on the same culture.

P. g. tritici under natural conditions, on cultivated plants of *Bromus coloratus*, *B. mollis*, *Hordeum distichon* and *H. murinum* has been reported earlier by Prasada (1951). *H. murinum* has been found susceptible to *P. g. tritici* in some other countries, such as Australia (Waterhouse, 1929), Argentine (Vallega, 1947), South Africa (Verwoerd, 1931) etc., and their findings have been substantiated during the course of these studies. Stakman and Premeisel (1917) could easily infect *A. elongatum* with *P. g. tritici* but *A. desertorum* and *A. sibiricum* gave negative results. The former two species of *Agropyron*, namely *A. desertorum* and *A. elongatum*, were found to be resistant to black rust of oat. These results have been confirmed except that *A. elongatum* and *A. sibiricum* were infected very weakly showing resistant types of pustules. *A. scabrum*, a perennial grass in Australia, reported to be susceptible to *P. g. tritici* in nature by Waterhouse (1929 and 1934) and also by Cass Smith and Millington (1944), was found to be moderately susceptible to black rust of wheat and fairly susceptible to yellow rust also.

Fischer and Claassen in U.S.A. (1944) inoculated 123 species of grasses in seedling stage by black rust from wild oats, identified as *P. graminis avenae*, and found that it could easily infect *Avena sativa* and *Bromus mollis*, the later being only mildly infected. In America, *Phalaris canariensis* is reported to be susceptible to *P. g. avenae* under field and glasshouse conditions. (Fischer and Levine, 1941). Susceptibility of other two species of Phalaris, namely *P. angusta* and *P. minor* has been reported from Argentina (Vallega, 1943). The results of other workers were confirmed during these studies except that *Phalaris canariensis* showed resistant type of pustules. In Australia Waterhouse (1929) found that *Lolium temulentum* is slightly infected by *P. g. avenae* whereas *Bromus mollis* and *Lolium temulentum* were found to be immune to *P. g. tritici* by him. In the present tests, however, *B. mollis* has been found to be highly susceptible to *P. g. tritici*. This difference may be due to the difference in the race flora of the two countries.

Yellow rust too can infect many grasses. Marchionatto (1931) has mentioned *Bromus unioloides* and *Hordeum jubatum* as collateral hosts of yellow rust from whence it can readily spread to wheat and Unamuno (1933) found *P. glumarum* infecting leaves of *Lolium perenne* and *L. rigidum* in Spain. Petrak (1941) from Greece recorded its occurrence on *Aegilops triuncialis* and Vallega (1947) in Argentina found that some species of wild *Hordeum* including *H. murinum* can be easily infected by yellow rust of wheat. In England Manners (1950) picked up race 33 of yellow rust on *H. murinum*, but *Bromus mollis* was found to be immune to yellow rust races in seedling stage. The findings of Petrak, Vallega and Manners were confirmed during the course of these studies. Inoculations of *Hordeum murinum* with *P. glumarum* yielded inconclusive results but as mentioned earlier, this plant has been found infected in nature by yellow rust of wheat.

Brown rust of wheat infects comparatively fewer grasses. Johnston (1940) tested some Agropyron species in the seedling stage and found that *Agropyron sibiricum* and *A. desertorum* were mildly infected by races 9 and 28 of *Puccinia triticina* (Eriks) but *A. pauciflorum* was not infected. We found that *Agropyron sibiricum* and *A. desertorum* were only weakly infected but *Aegilops triuncialis*, *Hordeum distichon* and *Bromus mollis* were found to be fairly susceptible to brown rust. Other species were either resistant

or immune. *Aegilops triuncialis* is reported to be susceptible in America also (Mains, 1933).

It will be seen from the foregoing account that some of the exotic grasses tested in seedling stage, were found to be fairly susceptible to one or more rusts. It is not unlikely, then that a few of them may get infected in nature by oat and wheat rusts if conditions are extremely favourable. In case, some of them get naturalised in the hills, where rusts survive during the critical period, they may play a significant part in the annual outbreak of the rusts in the plains of India. It is therefore, essential that all newly introduced exotic material be tested against Indian physiologic races of wheat rusts prior to their release for general cultivation in this country. Some foreign grasses *e. g. Vulpia myuros* and *Briza minor*, have already become firmly established in South India Hills and are severely infected by *Puccinia graminis avenae*. Indiscriminate introduction of grasses is therefore, likely to add to our difficulties regarding control of cereal rusts in India.

ACKNOWLEDGEMENT

Thanks are due to Dr. B. L. Chona, Systematic Mycologist, for supplying infected samples of grasses. Thanks are also due to Dr. N. Parthasarathy, Head of the Division of Botany and Mr. H. B. Singh for supplying the seeds of exotic grasses from their collection.

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CONTROL OF PAPAYA FOOT ROT

Rajpal Singh Nirvan

(Accepted for publication, September 19, 1953)

Foot rot of papaya is characterised by the appearance of water soaked patches on the stem at the ground level. These patches enlarge and ultimately girdle the entire circumference of the stem. On account of rotting, the diseased tissues become dark brown or black and in severe cases the plant topples over and dies. In less severe cases, the apical growth stops, lower leaves dry up and the fruits shrivel and fall to the ground. Subramaniam (1919) attributed this disease to *Pythium butleri* which was later changed to *Pythium aphanidermatum* (Eds.) Fitz.

During the monsoon season of 1950, 35% and 27% mortality due to this disease was observed at Alsibagh and Manjulkey gardens, Saharanpur. For controlling this disease Mitra (1932) recommends cutting away the infected areas at an early stage of the disease and disinfecting it by an antiseptic; whereas Mundkur (1949) has advised that papayas should always be planted in well drained soils and never in water logged ones. Both methods are generally impracticable for the orchardists. In this note are presented the results of a field experiment carried out for two years where Bordeaux mixture 6 : 6 : 50, was applied to the papaya stems as a protective spray.

The foot of the plant was sprayed after removing about two inches of soil all round it. The stem and the exposed soil were thoroughly drenched with the spray and the soil was again put round the stem. In all three sprays were given at an interval of twenty days, the first spray commencing with the appearance of rains in July. A papaya field with one and a half year old plants and in which the incidence of foot rot in the previous year was 35%, was selected for the experiment. The two treatments were Bordeaux spray and check. They were randomised in the field with three replications. One hundred and twenty papaya plants were sprayed and the same number kept as check. The number of casualities occuring due to the natural infection of foot rot, were recorded both for the spray and check plants.

The result of observations for the first year show that out of one hundred and twenty treated plants, the number of foot rot affected plants were seven as compared to twenty four in the check. Thus the percentage of the affected plants was six and twenty in the spray and check plants respectively in the first year, while it was 2·5 and 22·5 percent respectively in the second year of the experiment. In the second year the number of spraying was reduced to two with the addition of an sticker Alboleum to Bordeaux mixture. The rotting stems were examined and found to be affected with *Pythium aphanidermatum*.

Bordeaux mixture spray on papaya stem has thus retarded, consistently for two years, natural occurrence of foot rot disease to a considerable extent. A lesser incidence during the second year may be attributed

Fig. 1. Showing Foot root of a papaya plant.

to the effect of the Alboleum used as a sticker in combination with Bordeaux mixture. Stickers are known to enhance the protective qualities of fungicides by preventing their washing down during rains, Thus the addition of sticker has given a better control of foot rot as compared to Bordeaux mixture without it.

Though a hundred percent control could not be obtained by the method used, yet the results obtained through spray of Bordeaux mixture with sticker seem sufficient to recommend it as a practical solution for the orchardist to check the incidence of foot rot in the field.

My thanks are due to Dr. L. B. Singh, Horticulturist, for providing necessary facilities in carrying out this experiment.

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SOLAR ENERGY TREATMENT OF WHEAT LOOSE SMUT (*USTILAGO TRITICI* (PERS.) ROSTR.)

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(Accepted for publication, September 30, 1953)

Introduction

Loose smut is a serious disease of wheat. It occurs commonly wherever the crop is grown both in plains and hills. The incidence of attack is greater in comparatively moist than in dry areas.

The disease is very destructive as almost every head of the affected plants is converted into a black mass of spores and no grain is formed. In India the disease has significant economic importance as wheat is the main crop of the states of Punjab, Pepsu, Uttar Pradesh and Madhya Pradesh.

The area under wheat is nearly 23 million acres and the produce about 6·7 million tons. If the damage to grain is reckoned even at the low figure of 3 percent, monetary loss would amount to over 5 crores of rupees. But individual fields have been found to have 20-30 percent smutted heads in some States. A severe outbreak of loose smut varying from 3 to 30 percent was reported in 1951-52 from the Amritsar, Gurdaspur, Ludhiana, Hoshiarpur, Jullundur and Karnal Districts of the Punjab and damage to grain was estimated at 3 crores of rupees. The annual recurrence of such heavy losses calls for urgent measures to combat the disease. As explained in the context, the damage is preventable by the simple and effective solar energy treatment discovered* by the author in 1929. In this paper experiments leading to the development and standardisation of this method of control are described.

Life cycle of the causal Fungus:

Life cycle of the wheat loose smut fungus (*Ustilago tritici* (Pers.) Rostr.) and mode of perpetuation of the disease have been fully investigated. A brief description of it, however, is given here. The disease is recognisable only at the earing stage by conspicuous black heads which emerge earlier than normal healthy ones. Infection of the disease is carried inside the grain as dormant mycelium. After sowing of the grain the mycelium resumes growth and hyphae extend into tissues of the seedling. The activated fungus continues to grow keeping pace with the enlargement of the host without causing injury to it. At the time of earing, the fungus enters the reproductive phase forming numerous spores and disorganising the spikelets and embryo. Resting period of the fungus begins at this stage. Spores are blown away by wind and some falling on stigmas of blooming ears germinate there.

The germ tubes extending through the style enter the ovary. Development of the ovary into grain is not interfered with and proceeds

* The author was awarded the MAYNARD GANGARAM prize for this discovery in 1937 in a competition open to all countries.

as in non-infected flowers. The infected grain matures with the mycelium embodied in it and is identical with sound grain in appearance and function. The mycelium in dormant stage is lodged in the ripened grain and rests there. The produce of the crop is thus a mixture of sound and infected grains. On germination both kinds of grains produce plants apparently alike. Their difference is revealed on earing when the infected plants bear smutted heads. Spores restart the life history on attacking new plants through flowers (by blossom infection).

Experimental

It is since long that the disease has been known in India and foreign countries. Although effective and practicable control measures have been developed for several diseases, loose smut has presented peculiar difficulties. The causal fungus is enclosed in the grain as intraseminal mycelium and is thus not affected by surface killers and disinfectants. The use of general fungicides is, therefore, precluded for controlling the disease. Generally hand picking of smutted heads has been practised. But it would not appreciably reduce infection as spores are scattered before collection or even during gathering of black heads. Moreover, the method is laborious and does not appeal to farmers. In India this was the only method till recently applied for controlling the disease. Jensen (1889) of Denmark brought out the hot water treatment which was first used by Swingle (1892) against loose smut of wheat, and also by Freeman and Johnson (1909). The process consists of (1) soaking of wheat grain in ordinary cold water for 4 hours and (2) dipping in water at 132°F for ten minutes and finally drying the grain before sowing. The method has undergone several modifications since its discovery. A simpler method is as follows :—

Wheat is (1) soaked in ordinary water at 68-86°F for 4-6 hours, (2) placed in water at 120°F for 2 minutes, (3) immersed in water at 129°F for 10 minutes. The hot water methods have been employed where facilities of equipment and skilled help are easily provided.

Like the original Jensen's method, the somewhat simplified process also involves risk of injury to seed. Great care is needed to control temperature by a thermometer which is not within the means and capacity of most of the farmers to use.

Several attempts have been made to evolve a simple device suitable for operation by cultivators. Tapke (1924 and 1926) introduced single hot water immersion bath by application of steam. No doubt there is improvement in curtailing preliminary heating, but steam and other appliances cannot be available generally and this method also has limitations.

In India, Luthra and Sattar (1934) substituted the final immersion in water at 129°F for 10 minutes, by dipping grain in water at temperature ranging between 127°F and 132°F. The margin of 5 degrees made it easier to maintain the required lethal temperature and reduced chances of seed injury.

Another device of the same workers was to omit presoaking and treat the seed by single immersion in water at 115°F for 4 to 6 hours.

As an alternative to artificial heating, necessary temperature of the water bath was obtained by exposure to sun of a black painted galvanised vessel half filled with water. Temperature of water rose to 119°F after 9 hours' exposure. But none of these methods has found favour with cultivators.

This brief review of the progress of work on the subject led to the conclusion that the complication of heating water and provision of equipment for that purpose still left much room for improvement of the measures tried. Indian farmer is illiterate and cannot read thermometer and afford other requirements. He wants a method which he can apply unaided and with the least expense.

In view of the recurring heavy losses of wheat grain by this widespread disease, it was imperative to continue the investigation to evolve such a method of control as would fulfil the needs of farmers.

The author took up a new line of research with this objective. It was based on the fundamental physiological principle to the effect that protoplasmic structures like seed are rendered more susceptible to fatal injury by heat when saturated than in dry condition. The dormant mycelium of the smut fungus furnished very suitable subject for the study of the influence of heat therapy on living material. Experiments to explore this conception further were undertaken by the author in the Botanical Laboratory of the Punjab Agricultural College and Research Institute, Lyallpur (now in Pakistan) in 1929. Lyallpur is the largest wheat growing tract of the Chenab river colony. It has long hot summer registering temperature up to 120°F in shade. In the sun, temperature is over 130°F during May and June. On the black bulb vacuo-thermometer it may rise to 170°F. Wheat crop is harvested and thrashed under scorching rays of the sun in April-May. Wheat grains containing the dormant mycelium lie exposed for days at a stretch to the intense atmospheric heat. But the internal causal fungus is not harmed at all and the disease is reporduced in the wheat plant raised from the infected grain. The mycelium survives the adverse hot conditions and increasingly prepetuates the disease in the succeeding crop. It is inferred from these observations that wheat grain and the mycelium within it are highly resistant to dry heat of the sun.

Against this natural background, the effect of the solar energy on wheat soaked in water was studied. Keeping particularly in view the fate of internal mycelium to which moisture would penetrate, the following series of experiments combining varying periods of soaking and exposure to solar energy were conducted :

(*a*) 2 hours soaking and 1 hours exposure
(*b*) 3 hours soaking and 2 hours exposure
(*c*) 3 hours soaking and 3 hours exposure
(*d*) 4 hours soaking and 3 hours exposure
} Dried in shade after exposure to the sun.

(*e*) 4 hours soaking and 4 hours exposure or longer to dry the grain.

Soaking of grain was done in water contained in a bath tub in a room for the required period beginning at 8 A. M. and then spread out in single layer on a cloth or gunny sheeting and exposed to the sun for the necessary length of time.

Presoaking was considered an essential feature of the new method. Duration of immersion in water was intended to be long enough to let moisture penetrate thoroughly into the seed and wet the mycelium completely for operation of solar energy. The dormant mycelium is activated by moisture and thus rendered more vulnerable to solar action. Indication of adequate soaking is that the cut surface across the width of the treated grain, should appear uniformly moist without any white dry spot. Care, however, has to be taken to avoid softening of the grain by over-soaking. To determine the right period of soaking and sun heating the five combinations given above were tried. Improved wheat Type 8A (*Triticum vulgare*), a common bread wheat, was used for the treatments in 1929. Type 4 (*T. sphaerococcum*) a dwarf variety, was also included in later trials. Wheat seed was obtained from standing crop showing over 10 percent smutted heads. Germination of wheat seed was tested before and after the treatments. It was 98 to 100 percent. Samples treated under series (*a*) to (*d*) were dried in the shade to limit exposure to sun to the required time factor. Dried samples were stored after treatment in June. In November, 1929 they were sown in three replications with alternate plots of untreated seed as control. Observations were made on the emergence of ears in March 1930. Smutted heads appeared in all plots sown with seed soaked and exposed for less than 4 hours. The percentage of smutted heads varied from 4 to 10 calculated on counts of 100 plants. The crop of plot sown with seed soaked and exposed for 4 hours was absolutely free from the disease. The control plots had 5 to 15 percent of smutted heads. In view of encouraging results of the solar energy treatment in the first trial (Luthra, 1933) the experiments were continued. The seed of plots with over 10 percent smut was collected and stored for further trials under the successful series of 4 hours soaking and exposure. Seed was soaked in a tub of water from 8 A. M. to 12 noon in a room and then exposed to the sun up to 4 P. M. The remaining four treatments were dropped as they gave only partial control. Data of experiments carried out in 1931 and 1932 are given in Table I showing complete elimination of the disease. The incidence of attack in the untreated crop of Type 8A varied from 4·31 to 11·25 and that of Type 4 was 8·02 to 14·96. After corroboration of the efficacy of the solar energy treatment for three years at Lyallpur, large scale application of it was made on agricultural farms. In all cases the new method proved effective, and farmers who adopted it got rid of the disease on their holdings. The State Agricultural Department treated the seed of improved Types before distribution to growers.

The usefulness of the new method is in conformity with the views expressed in a Mycological Conference held in London in 1934. The criterion laid down was that "Control measures suitable for the small farmer must be reasonably efficient, exceptionally cheap and extremely simple and required materials and apparatus readily available. As long as any one of these essential requirements was not met, the method was useless". Results of trials carried out at Lyallpur, Sargodha and Jullundur farms in 1936-37 and 1937-38 are given in Table II. They confirm the efficacy of the new measure of control and also indicate a trend towards increase of yield. Further experiments conducted in July, August and September showed that the treatment would hold good even when the maximum shade temperature is near about 104°F provided there is bright sunshine for exposure. At the Gurdaspur Farm situated in

TABLE 1

Showing results of the application of Solar energy and percentage of smut before and after treatment

Serial No.	Type of wheat	Date of treatment	Duration of soaking in ordinary cold water	Duration of exposure to the sun (solar energy)	Temperature (°F) in the sun at 12 noon—4 P.M.		Maximum temperature (°F) in shade	Percentage germination	Percentage of smut
1	Pb.8A	10th June, 1931	A.M. 4 hours (8-12)	P.M. 4 hours (12-4)	122	129	114.2	100	0
	,,	Untreated	...	...	...	...	...	100	4.36
2	Pb.4	10th June, 1931	Do	Do	122	129	114.2	100	0
	,,	Untreated	...	...	...	...	...	100	8.02
3	Pb.8A	11th June, 1931	Do	Do	121	125	112.5	100	0
	,,	Untreated	...	...	...	...	...	100	4.31
4	Pb.4	11th June, 1931	Do	Do	121	125	112.5	100	0
	,,	Untreated	...	...	...	...	...	100	8.97
5	Pb.4	11th June, 1932	Do	Do	122	125	113	98	0
	,,	Untreated	...	...	...	...	...	100	13.09
6	Pb.4	27th June, 1932	Do	Do	121	125.5	112	99	0
	,,	Untreated	...	...	...	...	...	100	13.95
7	Pb.4	28th June, 1932	Do	Do	121	126	113	99	0.26
	,,	Untreated	...	...	...	...	...	100	13.04
8	Pb.8A	28th June, 1932	Do	Do	121	126	113	99	0
	,,	Untreated	...	...	...	...	...	100	9.70
9	Pb.4	6th July, 1932	Do	Do	123	124.5	113	98	0
	,,	Untreated	...	...	...	...	...	100	14.96
10	Pb.8A	6th June, 1932	Do	Do	123	124.5	113	98	0
	,,	Untreated	...	...	121	125.5	...	100	11.25
11	Pb.8A	27th June, 1932	Do	Do	121	125.5	112	98	0
	,,	Untreated	...	...	...	...	...	100	10.85

TABLE II

Showing percentage smut infection and grain yield of wheat crop grown at some farms after treatment of the seed with solar energy

PLACE	Plot Area (Acres)	Replications (No.)	INFECTION (%)		YIELD (lbs)		Remarks
			Control	Treated	Control	Treated	
Lyallpur-Mycological Area (1936-37)	1/50	8	13.5	Nil	28.32	29.08	In all cases the treatment is effective and shows a trend towards increase in yield.
Botanical Farm (1937-38)	1/41	6	15.2	,,	58.80	60.40	
Sargodha-Agricultural Farm (1936-37)	1/25	5	16.2	,,	57.08	61.4	
Jullundur-Agricultural Farm (1936-37)	1/25	5	14.4	,,	64.40	76.80	

submontane tract a trial was made in July, 1931, on a clear day with shade temperature of 102°F only. The crop of treated seed showed only 0·3 percent of the disease as against 18 percent of the untreated control thus showing that only negligible infection remained uncontrolled. The Imperial Mycologist I. A. R. I. found the method successful under North Bihar conditions, as obtained at Pusa. The solar energy treatment has been taken up also in Pepsu, Uttar Pradesh and Madhya Pradesh. In the Punjab, on account of dislocation of work by partition of the country in 1947, the treatment, previously regularly applied, somewhat fell into abeyance, but it has been resumed now and 15,000 maunds of wheat were reported to have been treated in 1951-52.

Extension of solar energy treatment to other smut diseases : In Burma, Seth (1943) applied this method to milo-(millet) grain smut and found it effective. Asthana (1947) found the control of covered smut of sorghum (*Spacelotheca sorghi* (Link) Clinton) by solar energy as effective as chemical treatment. Vasudeva and Iyengar (1950) tried it for barley loose smut and found it effective. Patel *et al* (1950) got similar results on millet grain smut at Poona. Mehta (1951) also applied it with success to barley covered smut in U. P. United Nations Scientific Department brought the solar energy process to the notice of member countries and Turkey has commended it as best suited to her climatic conditions for controlling wheat loose smut.

Help of S. Riswal Singh and Dr. Kishan Singh Bedi in field work is gratefully acknowledged.

Summary

1. Wheat loose smut (*Ustilago tritici* (Pers.) Rostr.) is reported to be wide-spread in India causing damage to grain amounting to over 5 crores of rupees annually.

2. Work done abroad and in India on hot water treatment as a control measure of the disease is reviewed.

3. Technique of the new solar energy treatment is described giving details of experiments carried out and results obtained :

The method consists of two steps :

(*i*) Presoaking of grain in water for 4 hours in shade or in a room.

(*ii*) Exposure of the soaked grain to the sun for 4 hours to let solar energy operate on the intraseminal mycelium and also dry the grain.

4. Application of solar energy to smut diseases of other crops is reported.

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BLIGHT OF CYPERUS ROTUNDUS L. AND C. BULBOSUS VAHL

KUMARI V. SEETHALAKSHMI

(Accepted for publication, October 1, 1953)

Cyperus rotundus and *C. bulbosus*, two of the common sedges are subject to the incidence of blight disease during the rainy months of October and November in and around Coimbatore. The infection is evident by the formation of brown watersoaked lesions on the leaf-blade and leaf-sheath. Eventually the aerial shoots wither and die. The spread of infection is however arrested on the cessation of the rains. Of the two species of *Phytophthora* associated with this blight, one was identified as *P. cyperi-rotundati* Sawada and the other was found to be new and described as *P. cyperi-bulbosi* Seethalakshmi and Ramakrishnan (1953).

Kawakamia was established as a genus of Peronosporaceae having as its type fungus, *K. cyperi* (Miyabe and Ideta) Miyabe, infecting *C. tegetiformis* Roxb. in Japan. This fungus had close resemblance to *Phytophthora* but differed from it in being an obligate parasite. Sahaya (1936) described a similar disease from Assam and Bihar in India on the same host. Tokunaga (1935) revised this as *P. cyperi* (Ideta) S. Ito. While Wilson (1914) is of opinion that this genus is closely allied to *Basidiophora*, Fitzpatrick (1930) is of the view that there are no valid grounds for separating *Kawakamia* from *Phytophthora*. Another species of *Kawakamia* viz., *K. carica* Hara originally recorded on fig was later revised as *Phytophthora carica* (Hara) Hori (Tanaka, 1920). Which was later considered a synonym of *P. palmivora* by Tucker (1931). Sawada revised *P. colocasiae* as *K. colocasiae* but at the present time the former name only is recognised. *Phytophthora cyperi* (Ideta) S. Ito, on *C. malaccencis* Lam. and *C. tegetiformis*, *P. cyperi-rotundati* Sawada on *C. rotundus* and *P. cyperi iriae* Sawada on *C. iria* L have been recorded as parasitic on the genus *Cyperus* from Japan and Formosa. Thus four closely allied fungi have been observed on this host genus. Of the two fungi observed at Coimbatore one is identified as *P. cyperi-rotundati* while the other has been named *P. cyperi-bulbosi* owing to certain specific differences. These two are described in this communication.

P. cyperi-rotundati

This is common on *Cyperus rotundus* and occassionally on *Cyperus bulbosus*. In the initial stages one or more watersoaked brownish lesions develop on the leaf blades. These spread rapidly destroying the leaves and causing the death and decay of the whole shoot. The disease resulted in the death of several plants in widening patches.

The mycelium is intercellular. In the rainy season, mildewy growth is evident on the lower surface of the leaves. This consists of sporangiophores and sporangia. The former come out through the stomata and bear one or more obpyriform sporangia. The sporangia are thin walled with broad papilla and measure $42-57 \times 27-33\mu$. Detached sporangia have a persistent stalk upto 8μ in length. The germination takes place either by the production of a germ tube from the papillary end or by the formation of zoospores.

Numerous oospores crowding the air spaces between the veins develop in the tissues of the leaf. The oogania are subglobose, smooth, yellowish brown in colour and measure $27-45\mu \times 27-39\mu$. The oospores which do not completely fill the oogania, are thick walled, yellowish brown and spherical, measuring $24-33\mu$. Each ooganium has one persistent antheridium. In the majority of cases the antheridium is amphigynous though rarely a few are found to be paragynous.

The fungus defied all attempts to bring it into pure culture on any of the agar media. Its pathogenicity was tested by inoculating the healthy leaves of *C. rotundus* and *C. bulbosus*, placing drops of water containing sporangia removed from infected leaves. Successful infection was obtained in 3 to 4 days, as evidenced by the development of watersoaked spots.

The fungus prevalent in Coimbatore agrees closely with *P. cyperi-rotundati* and is therefore identified as such. Though it has not been possible to bring this fungus into culture, it need not be considered as a reason for excluding it from the genus *Phytophthora*. As the technique improved, some fungi which were originally considered to be obligate parasites have been grown on artificial media (e.g. *Empusa* and smuts). Therefore it is not proper to consider this distinction alone as good enough for a generic differentiation and establishing the genus *Kawakamia*.

P. cyperi-bulbosi

This fungus is confined to *C. bulbosus*. The symptoms of infection are similar to those produced by *P. cyperi-rotundati* and the plants dry up in case of severe infection. The mycelium permeates the tissues of the leaf-blade and sheath intercellularly. Slender sporangiophores emerge through the stomata bearing terminal obpyriform sporangia with broad or obtuse papilla. Sympodial branching of the stalks is observed in some cases leading to the development of 4 to 5 sporangia on a branched sporangiophore. These varied in size averaging $40 \times 25\mu$ ($23-50 \times 20-34$). When these are floated in drops of water, germination takes place in about half an hour. Direct germination by the formation of a germ tube or indirect germination resulting in zoospore development may follow.

Numerous oospores are formed in the leaf-sheaths in air chambers of the mesophyll. Sometimes they may be visible in lines inside the tissues. The oogonia are spherical and persistent becoming dark brown and developing tuberculated thickenings on the wall. In sectional view, the thickenings stand out clearly. The surface view presents a reticulated appearance. The average diameter is 40μ ($31-53$). The antheridia are hemispherical, paragynous, hyaline and persistent. One antheridium is usually attached to each ooganium but rarely two may also be observed. The oospores are spherical and invariably plerotic. They are thickwalled, smooth and brown. On an average they are 33μ ($25-42$). Mature oospores germinate readily without a resting period. They put forth short germ tubes in seventy two (72) hours. These germ tubes bear terminal sporangia of the same shape and size as the normal sporangia.

Repeated attempts made to bring the fungus into culture from infected tissues and from the sporangia were unsuccessful. Therefore all infection experiments had to be carried out with the sporangia obtained

from naturally infected leaves. Suspensions containing the sporangia in water were placed on the leaves of healthy plants of *C. bulbosus* and *C. rotundus* grown in pots. The inoculated plants were kept under bell jars for seventy two (72) hours. By this time, lesions developed on the inoculated leaves of *C. bulbosus* alone while the controls and *C. rotundus* remained unaffected. The experiments were repeated and the results confirmed.

This fungus exhibited differences from *P. cyperi-rotundati* and others reported on *Cyperus*, in possessing tuberculate oogonia and exclusively paragynous antheridia.

Since both these sedges are troublesome weeds in cultivated lands, any specific disease which will destroy them will be advantageous to the farmer. But the blight described in this paper though capable of destroying the aerial shoots during the rains does not extend to the underground tubers and is not helpful in eradicating the weed. Once the rains abate, the spread of infection is arrested.

Summary

Blight caused by *Phytophthora cyperi-rotundati* on *Cyperus rotundus* and *C. bulbosus* and by *P. cyperi-bulbosi* on *C. bulbosus* alone is prevalent in coimbatore during the rainy season. These do not grow on artificial media unlike other species of *Phytophthora.*

Acknowledgement

My grateful thanks are due to Sri T. S. Ramakrishnan, M.A., F.A.Sc., Government Mycologist, Coimbatore for help rendered in the preparation of this article.

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Explanation of Plates

Plate I

Fig. 1. Diseased leaves of *Cyperus rotundus* showing lesions on leaf blades.

Fig. 2. Sporangia of *P. cyperi rotundati* with persistent stalks.

Fig. 3. Oospores of *P. cyperi-rotundati* with amphigynous antheridia.

Fig. 4. Sporangium of *P. cyperi-bulbosi*

Fig. 5. Oospores of *P. cyperi-bulbosi*

Plate II

P. cyperi-bulbosi

Fig. 1. Sporangium after the liberation of zoospores.

Fig. 2. Sympodial branching.

Fig. 3. Germinating sporangium (conidial).

Fig. 4. Sporangiophore bearing cluster of sporangia.

Fig. 5. Oospore with a paragynous antheridium.

Fig. 6. Germinating oospore with a sporangium at the end of the stalk.

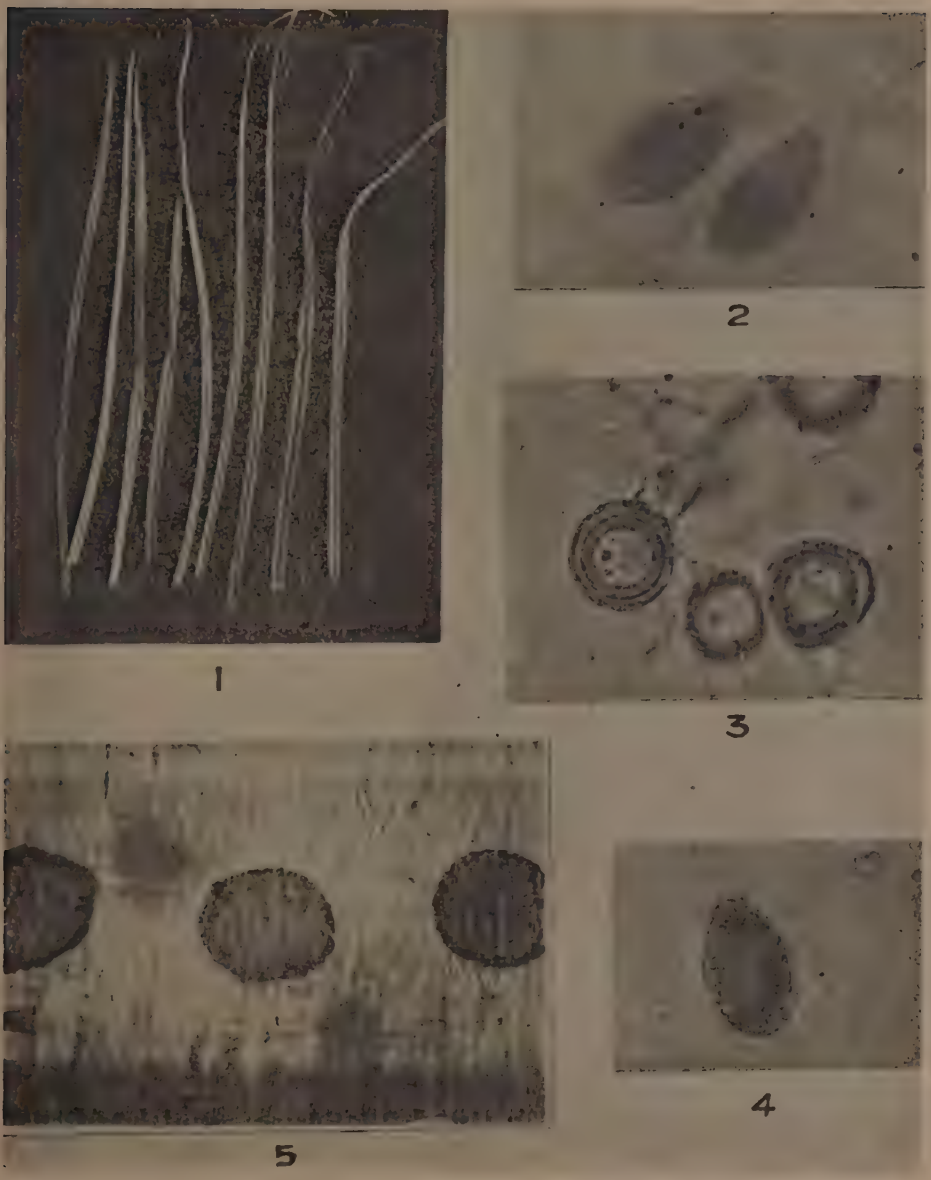

PLATE I

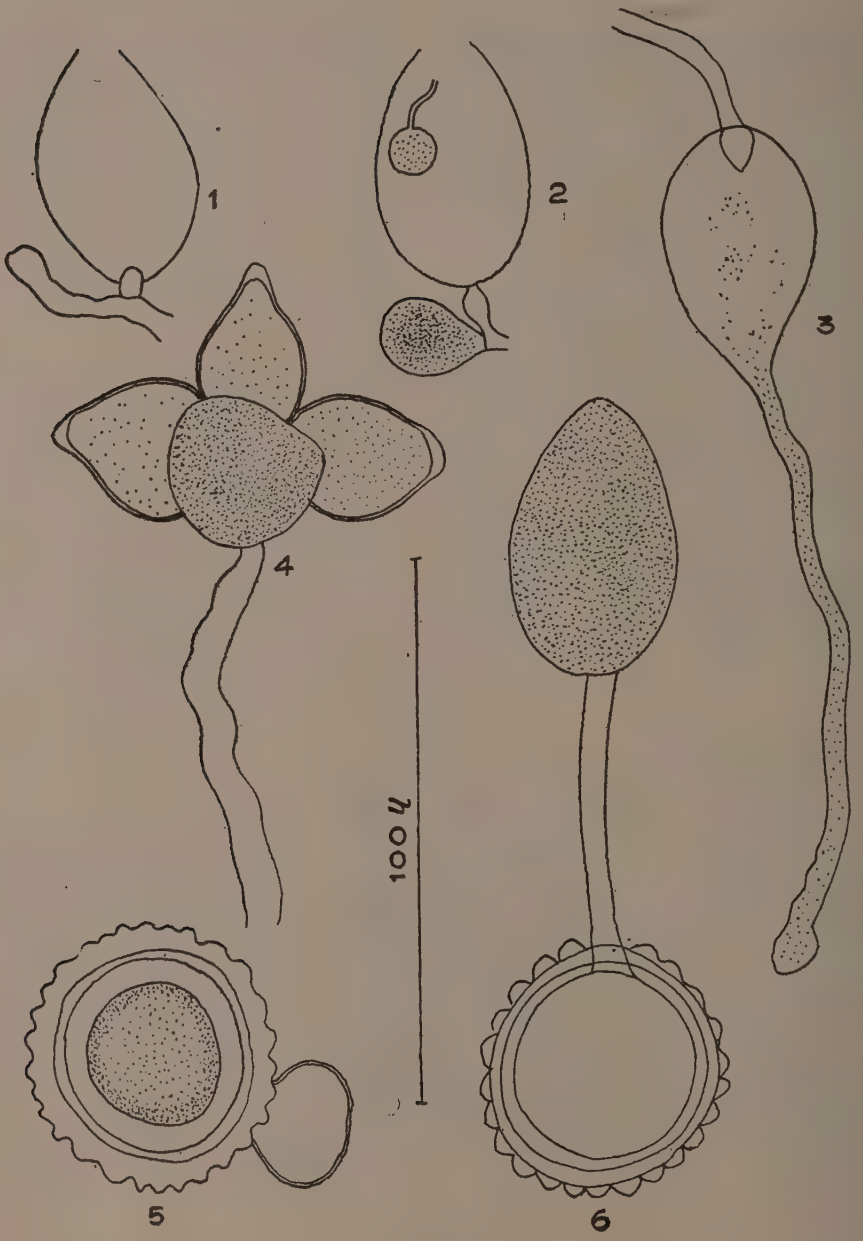

PLATE II

OCCURRENCE IN NATURE OF *PHYSALOSPORA TUCUMANENSIS* SPEG., THE PERFECT STAGE OF SUGARCANE RED ROT ORGANISM, IN INDIA

B. L. Chona and B. S. Bajaj

(Accepted for publication, October 4, 1953)

On a routine examination of an 18 months old sugarcane crop at New Delhi, some dried up old leaves of various cane varieties still attached to the plant, showed numerous, small, black dots on the leaf lamina, and to a lesser extent on leaf sheaths, resembling pycnidia or perithecia. On microscopic examination they were found to agree with the recorded description of *Physalospora tucumanensis*, which has been reported to be the perfect stage of *Colletotrichum falcatum* Went by Carvajal and Edgerton (1944) from U. S. A. and Ling and Ma (1950) from Formosa. In India the natural occurrence of this stage has not so far been recorded although a good deal of work has been done on the disease and its causal organism since 1906. Recently, Chona and Srivastava (1952) have reported the production of the ascigerous stage of *C. falcatum* in culture under laboratory conditions. Preliminary morphologic studies of this fungus as observed in its natural habitat are reported here.

The *perithecia* are found scattered as small, black dots on the leaf lamina, being more abundant on the dorsal side (Plate I, Fig. 1) completely submerged in the host tissue with a small ostiole protruding to the outside. Perithecia are usually located between the fibrovascular bundles and can be easily separated from the substratum by simple teasing (Plate I, Fig. 2). They measure 160-300 μ (average 231·4 μ) in width; and 105-210 μ (average 156·5 μ) in height, and contain numerous asci and paraphyses. *Asci* are clavate, tapering towards the lower end and have a hyaline wall (Plate I, Fig. 3). They vary in size from 43·5 to 73·5 μ (average 62·5 μ) in length and 7·0 to 13·5μ (average 10·8 μ) in width. *Ascospores*, eight in number in each ascus, are arranged in two rows (biseriate). These are single celled, hyaline, elliptical to ovate, with both ends obtuse, and contain one or more vacuoles (upto four seen). They measure 16—23 $\times$ 5—7·5μ with an average size of 18·4 $\times$ 5·8μ (Fig. 5). The *paraphyses* are abundant, filled with conspicuous granules or oil droplets.

Ascospores germinate readily at Room temperature (31-33°C) in tap water. Single-ascus cultures made on oat meal agar and incubated at Room temperature (31-33°C) gave typical growth of *Colletotrichum falcatum* and produced setae and pinkish masses of spores by the 5th day. This culture closely resembled light type, freely sporing isolates of *C. falcatum*. The *conidia* are hyaline, unicellular, falcate with one oil globule and measure 19-30μ with an average of 25·7μ. The conidia are borne on conidiophores in acervuli with setae (Plate I, Fig. 4). The *acervuli* are black stromatic masses. *Setae* are dark brown in colour, pointed, thickened below and tapering towards the apex, measuring 77-154μ.

The above studies clearly prove the connection of the ascigerous stage now obtained in nature with the conidial stage (*C. falcatum*). The asci and ascospores in our case are slightly smaller in size than those originally

Fig. 1

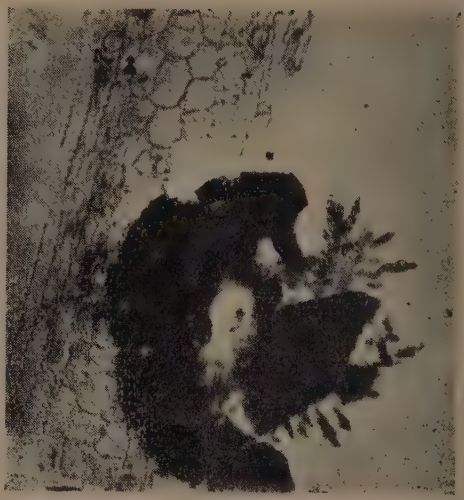

Fig. 2

Fig. 1—Sugarcane leaves showing abundant, dot-like perithecia of *Physalospora tucumanensis*. (a) Dorsal surface. (b) Ventral surface.

Fig. 2—A ruptured perithecium of *P. tucumanensis*, partly attached to the host tissue. Asci are seen emerging out. (X 230)

Fig 3

Fig. 4

PLATE I

Fig. 3—A mass of asci from a perithecium stained with Cotton Blue. (X 400)

Fig. 4—Acervuli of *Colletotrichum falcatum* obtained from the culture of an ascus of *P. tucamanensis* (X 400)

described by Spegazzini (1896) or later reported by Carvajal and Edgerton (1944) but fall well within the range of spore size stated by them. The fungus has, therefore, been identified as *Physalospora tucumanensis* Speg. Further work on the pathogenicity of this fungus is in progress, with a view to determine the role of *Physalospora* stage in the origin of new, more virulent strains of the Red Rot fungus, which bring about, off and on, serious Red Rot epidemic, and thus prove a great menace to the Sugar Industry of the country.

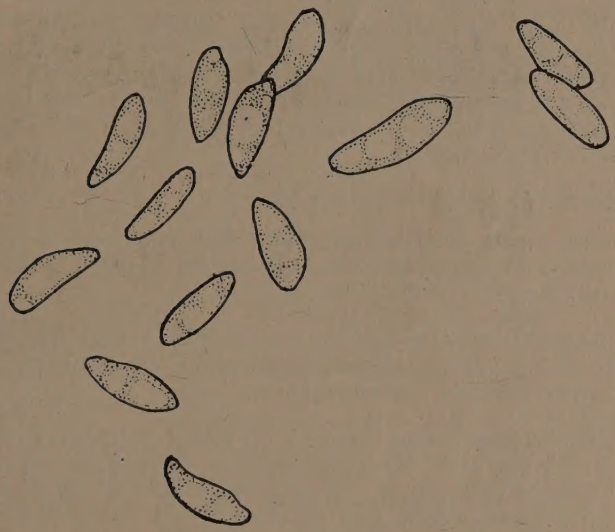

Fig. 5—Ascospores showing vacuoles. (X 800)

Our grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi for his keen interest and helpful criticism and for providing the necesary facilities, as also to Mr. Ram Lal Munjal, Assistant Mycologist, for critical examination of the manuscript and some of the slides.

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Indian Agricultural Research Institute,
New Delhi-12.

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Published & Edited by R. S. Vasudeva for the Indian Phytopathological Society
Pusa Buildings, New Delhi-12
and Printed at Janta Press, New Delhi